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(21) International Application Number: PCT/US98/21349 (22) International Filing Date: 8 October 1998 (08.10.98) (30) Priority Data: 60/062,109 14 October 1997 (14.10.97) US (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US 60/062,109 (CON) Filed on 14 October 1997 (14.10.97) (71) Applicant (for all designated States except US): CAMBRIDGE NEUROSCIENCE, INC. [US/US]; Building 700, One Kendall Square, Cambridge, MA 02139 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): MCBURNEY, Robert, N. [US/US]; 20 Leslie Road, Newton, MA 02166 (US). HOLT, William [US/US]; 162 A East Central Street, Natick, MA 01760 (US). GWYNNE, David, I. [US/US]; 77 Grover Street, Beverly, MA 01915 (US). MARCHIONNI, Mark [US/US]; 24 Twin Circle Drive, Arlington, MA 02174 (US).		(74) Agents: CONLIN, David, G. et al.; Dike, Bronstein, Roberts & Cushman, LLP, 130 Water Street, Boston, MA 02109 (US). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: THERAPEUTIC METHODS COMPRISING USE OF A NEUREGULIN (57) Abstract The invention provides methods for treatment and/or prophylaxis of certain neurological-related disorders, particularly treatment or prophylaxis of the effects of stroke, brain or spinal cord injury or ischemia, heart attack, optic nerve and retinal injury and ischemia and other acute-type conditions disclosed herein as well as chronic-type conditions, specifically epilepsy, Alzheimer's disease, Parkinson's disease, Huntington's disease, Amyotrophic Lateral Sclerosis, Down's Syndrome, Korsakoff's disease, cerebral palsy and/or age-dependent dementia. The methods of the invention comprise administration of a neuregulin, or fragment or derivative of a neuregulin, or a nucleic acid encoding a neuregulin or a neuregulin fragment or derivative, to a patient suffering from or susceptible to such conditions.		

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THERAPEUTIC METHODS COMPRISING USE OF A NEUREGULIN

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to methods for treatment of certain neurological-related injuries and disorders comprising use of a neuregulin, or a fragment or derivative of a neuregulin, or a nucleic acid encoding a neuregulin or neuregulin fragment or derivative.

2. Background

Nerve cell death (degeneration) can cause potentially devastating and irreversible effects for an individual and may occur e.g. as a result of stroke, heart attack or other brain or spinal chord ischemia or trauma. Additionally, neurodegenerative disorders involve nerve cell death (degeneration) such as Alzheimer's disease, Parkinson's disease, Huntington's disease, Amyotrophic Lateral Sclerosis, Down's Syndrome and Korsakoff's disease.

Therapies have been investigated to treat nerve cell degeneration and related disorders, e.g., by limiting the extent of nerve cell death that may otherwise occur to an individual as well as promoting repair, remodeling and reprogramming after stroke or other neuronal injury. See, e.g., F. Seil, *Curr Opin Neuro*, 10:49-51 (1997); N. L. Reddy et al., *J Med Chem*, 37:260-267 (1994); and WO 95/20950.

Certain growth factors have been reported to exhibit neuroprotective properties. In particular, nerve growth factor (NGF) has been evaluated in certain neuroprotective models. See, for example, G. Sinson et al., *J Neurosurg*, 86(3):511-518 (1997); and G. Sinson et al., *J Neurochem*, 65(5):2209-2216 (1995). Osteogenic protein-1 (OP-1) has been evaluated in a rat model of cerebral hypoxia/ischemia for neuroprotective activity. G. Perides, *Neurosci Lett*, 187(1):21-24 (1995). Glial cell line-derived neurotrophic factor (GDNF) was reported to exhibit trophic activity on certain populations of central neurons. Y. Wang et al., *J Neurosci*, 17(11):4341-4348 (1997). Small molecules also have been investigated as neuroprotective agents, such

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as MK-801. See B. Meldrum, *Cereb Brain Metab Rev*, 2:27-57 (1990); D. Choi, *Cereb Brain Metab Rev*, 2:27-57 (1990).

However, no effective pharmacotherapies are in regular clinical use for ischemia-induced brain injury or other such injuries and disorders. See, for example,
5 Y. Wang et al., *supra*; G. Sinson et al., *J Neurochem*, *J Neurochem*, 65(5):2209 (1995).

It thus would be highly desirable to have new neuroprotective agents, particularly agents to limit the extent or otherwise treat nerve cell death (degeneration) that occur with stroke, heart attack or brain or spinal cord trauma, or to treat
10 Alzheimer's disease, Parkinson's disease, Huntington's disease, Amyotrophic Lateral Sclerosis, Down's Syndrome and Korsakoff's disease. It also would be desirable to have agents that promote repair, remodeling or reprogramming after stroke or other neuronal injury.

SUMMARY OF THE INVENTION

15 The present invention provides methods for treatment and/or prophylaxis of certain neurological-related disorders, particularly treatment or prophylaxis of the effects of stroke, brain or spinal cord injury or ischemia, heart attack, optic nerve and retinal injury and ischemia and other acute-type conditions disclosed herein as well as chronic-type conditions, specifically epilepsy, Alzheimer's disease, Parkinson's
20 disease, Huntington's disease, Amyotrophic Lateral Sclerosis, Down's Syndrome, Korsakoff's disease, cerebral palsy and/or age-dependent dementia. Methods of the invention also include therapies for promoting repair, remodeling or reprogramming after stroke or other neuronal injury.

The methods of the invention comprise administration of an effective amount
25 of neuregulin, or fragment or derivative of a neuregulin, or a nucleic acid encoding a neuregulin or a neuregulin fragment or derivative (i.e. gene therapy), to a patient suffering from or susceptible to such conditions.

Neuregulins are members of the epidermal growth factor (EGF) superfamily and include glial growth factor (GGF), acetylcholine receptor-inducing activity
30 (ARIA), neu differentiation factor (NDF) and heregulins (HRF). See D. E. Wen et al., *Cell*, 69:559-572 (1992); W.E. Holmes et al., *Science*, 256:1205-1210 (1992); M.A. Marchionni et al., *Nature*, 362:312-318 (1993); and D.L. Falls, *Cell*, 72:801-815

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(1993). A variety of neuregulins and fragments and derivatives thereof can be employed in the methods of the invention. For example, suitable agents have been disclosed in U.S. Patent 5,530,109 and PCT/US93/07491. Neuregulins also have been reported in U.S. Patent 5,367,060. Preferred neuregulins include regions shown in FIGS. 1-2 (SEQ ID NOS. 2 and 4), also known as the E sequence. Preferred neuregulins or fragments or derivatives also include those that contain the C, C/D or C/D' sequences as shown in Figures 7, 8 and 9 respectively of the drawings, or those neuregulins or fragments or derivatives that have substantial homology to the peptide sequences shown in Figures 7, 8 or 9, e.g. at least about 70 percent homology, or at least about 80 percent homology, or more preferably at least about 90 or 95 percent homology to the peptide sequences shown in Figures 7, 8 or 9. Preferred nucleic acids and fragments and derivatives for use in the methods of the invention include those nucleic acids that include one or more nucleic acids sequences shown in Figures 7, 8 and 9 of the drawings, or those nucleic acids that that have substantial homology to the nucleic acid sequences shown in Figures 7, 8 or 9, e.g. at least about 70, 80, 90 or 95 percent homology to the nucleic acid sequences shown in Figures 7, 8 or 9. A particularly preferred neuregulin is encoded by DNA obtainable from the clone pGGF2HBS11 (ATCC Deposit No. 75347). Also preferred are neuregulins encoded by DNA obtainable from GGF2BPP5, GGF2BPP2 and GGF2BPP4.

Typical patients that may be treated in accordance with the methods of the invention are persons suffering from brain or spinal cord trauma or ischemia, stroke, heart attack, hypoxia, hypoglycemia, post-surgical neurological deficits, decreased blood flow or nutrient supply to retinal tissue or optic nerve, retinal trauma or ischemia or optic nerve injury. Patients suffering from chronic-type conditions also may be treated in accordance with the invention, specifically subjects suffering from or susceptible to epilepsy, Parkinson's disease, Huntington's disease, Amyotrophic Lateral Sclerosis, Alzheimer's disease, Down's Syndrome, Korsakoff's disease, cerebral palsy and/or age-dependent dementia.

Also, as discussed above, a neuregulin or fragment or derivative thereof or nucleic acid encoding same, may be administered to promote repair, remodeling or reprogramming to a subject that has suffered stroke or other neuronal injury such as traumatic brain or spinal cord injury. In such cases, the therapeutic agent may be

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suitably administered to the subject over an extended period following the injury, e.g. at least about 1, 2, 3, 4, 6, 8, 12 or 16 weeks following the injury.

Other aspects of the invention are disclosed *infra*.

BRIEF DESCRIPTION OF THE DRAWINGS

5 FIG. 1 shows a nucleotide sequence (SEQ ID NO:1) encoding a preferred neuregulin region (E segment of human GGF) and the amino acid sequence (SEQ ID NO:2) of that preferred region.

FIG. 2 shows a nucleotide sequence (SEQ ID NO:3) encoding a preferred neuregulin region (E segment of bovine GGF) and the amino acid sequence (SEQ ID
10 NO:4) of that preferred region.

FIG. 3 shows nucleotide sequences (SEQ ID NOS:6-7) encoding further neuregulin regions (B segment of human and bovine GGF) and amino acid sequences (SEQ ID NOS:5 and 8) of those regions. Line 1 is the predicted amino acid sequence of bovine B segment, line 2 is a nucleotide sequence of bovine B segment, line 3 is a
15 nucleotide sequence of human B segment (nucleotide base matches are indicated with a vertical line), and line 4 is the predicted amino acid sequence of human B segment shown where it differs from the bovine sequence set forth in line 1 of the figure.

FIG. 4 shows nucleotide sequences (SEQ ID NOS:10-11) encoding further neuregulin regions (A segment of human and bovine GGF) and amino acid sequences
20 (SEQ ID NOS:9 and 12) of those regions. Line 1 is the predicted amino acid sequence of bovine A segment, line 2 is a nucleotide sequence of bovine A segment, line 3 is a nucleotide sequence of human A segment (nucleotide base matches are indicated with a vertical line), and line 4 is the predicted amino acid sequence of human A segment shown where it differs from the bovine sequence set forth in line 1
25 of the figure.

FIG. 5 shows a nucleotide sequence (SEQ ID NO:13) encoding a further neuregulin region (A' segment of bovine GGF) and the predicted amino acid sequence (SEQ ID NO:14) of that region.

FIG. 6 shows nucleotide sequences (SEQ ID NOS:16-17) encoding further
30 neuregulin regions (G segment of bovine and human GGF) and amino acid sequences (SEQ ID NOS:15 and 18) of that region. Line 1 is the predicted amino acid sequence of bovine G segment, line 2 is a nucleotide sequence of bovine G segment, line 3 is a

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nucleotide sequence of human G segment (nucleotide base matches are indicated with a vertical line), and line 4 is the predicted amino acid sequence of human G segment shown where it differs from the bovine sequence set forth in line 1 of the figure.

FIG. 7 shows nucleotide sequences (SEQ ID NOS:20-21) encoding further
5 neuregulin regions (C segment of bovine and human GGF) and amino acid sequences (SEQ ID NOS:19 and 22) of those regions. Line 1 is the predicted amino acid sequence of bovine C segment, line 2 is a nucleotide sequence of bovine C segment, line 3 is a nucleotide sequence of human C segment (nucleotide base matches are indicated with a vertical line), and line 4 is the predicted amino acid sequence of
10 human C segment shown where it differs from the bovine sequence set forth in line 1 of the figure.

FIG. 8 shows nucleotide sequences (SEQ ID NOS:24-25) encoding further neuregulin regions (C/D segment of human and bovine GGF) and amino acid sequences (SEQ ID NOS:23 and 26) of those regions. Line 1 is the predicted amino
15 acid sequence of bovine C/D segment, line 2 is a nucleotide sequence of bovine C/D segment, line 3 is a nucleotide sequence of human C/D segment (nucleotide base matches are indicated with a vertical line), and line 4 is the predicted amino acid sequence of human C/D segment shown where it differs from the bovine sequence set forth in line 1 of the figure.

FIG. 9 shows nucleotide sequences (SEQ ID NOS:28-29) encoding a further neuregulin region (C/D' segment of the human and bovine GGF) and the amino acid sequence (SEQ ID NO:27) of that region. Line 1 is the predicted amino acid sequence of the C/D' segment, line 2 is a nucleotide sequence of bovine C/D' segment and line
20 3 is a nucleotide sequence of human C/D' segment (nucleotide base matches are indicated with a vertical line).

FIG. 10 shows nucleotide sequences (SEQ ID NOS:31-32) encoding a further neuregulin region (D segment of the human and bovine GGF) and the amino acid sequence (SEQ ID NO:30) of that region. Line 1 is the predicted amino acid sequence of the D segment, line 2 is a nucleotide sequence of bovine D segment and line 3 is a
30 nucleotide sequence of human D segment (nucleotide base matches are indicated with a vertical line).

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FIG. 11 shows nucleotide sequence (SEQ ID NO:34) encoding a further neuregulin region (D' segment of bovine GGF) and the amino acid sequence (SEQ ID NO:33) of that region.

FIGS. 12A-12B show nucleotide sequences (SEQ ID NOS:36-37) encoding further neuregulin regions (H segment of human and bovine GGF) and amino acid sequences (SEQ ID NO:35 and 38) of that region. Line 1 is the predicted amino acid sequence of bovine H segment, line 2 is a nucleotide sequence of bovine H segment, line 3 is a nucleotide sequence of human H segment (nucleotide base matches are indicated with a vertical line), and line 4 is the predicted amino acid sequence of human H segment shown where it differs from the bovine sequence set forth in line 1 of the figure.

FIG. 13 shows a nucleotide sequence (SEQ ID NO:40) encoding a further neuregulin region (K segment of bovine GGF) and the amino acid sequence (SEQ ID NO:39) of that region.

FIGS. 14A-14C show nucleotide sequences (SEQ ID NOS:42-43) encoding a further neuregulin region (L segment of bovine and human GGF) and amino acid sequences (SEQ ID NO:41 and 44) of that region. Line 1 is the predicted amino acid sequence of bovine L segment, line 2 is a nucleotide sequence of bovine L segment, line 3 is a nucleotide sequence of human L segment (nucleotide base matches are indicated with a vertical line), and line 4 is the predicted amino acid sequence of human L segment shown where it differs from the bovine sequence set forth in line 1 of the figure.

FIG. 15 shows nucleotide sequences (SEQ ID NOS:46-47) encoding further neuregulin regions (F segment of bovine and human GGF) and amino acid sequences (SEQ ID NOS:45 and 48) of that region. Line 1 is the predicted amino acid sequence of bovine F segment, line 2 is a nucleotide sequence of bovine F segment, line 3 is a nucleotide sequence of human F segment (nucleotide base matches are indicated with a vertical line), and line 4 is the predicted amino acid sequence of human F segment shown where it differs from the bovine sequence set forth in line 1 of the figure.

FIGS. 16A-16C show the nucleotide sequence (SEQ ID NO:49) and deduced amino acid sequence (SEQ ID NO:50) of GGF2BPP4.

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FIGS. 17A-17B show the nucleotide sequence (SEQ ID NO:51) and deduced amino acid sequence (SEQ ID NO:52) of GGF2BPP2.

FIGS. 18A-18B show the nucleotide sequence (SEQ ID NO:53) and deduced amino acid sequence (SEQ ID NO:54) of GGF2BPP5.

5 DETAILED DESCRIPTION OF THE INVENTION

As discussed above, preferred neuregulins for use in the therapeutic methods of the present invention include those disclosed in U.S. Patent 5,530,109 and PCT/US93/07491, incorporated herein by reference. Particularly preferred neuregulins comprise an amino acid sequence of the following formula:

10 WYBAZCX

wherein WYBAZCX is composed of amino acid sequences that include one or more sequences shown in FIGS. 1 through 15 (which includes SEQ ID NOS:2, 4, 5, 8, 9, 12, 14, 15, 18, 19, 22, 23, 26, 27, 30, 33, 35, 38, 39, 41, 44, 45 and 48), wherein W comprises the polypeptide segment F, or is absent; wherein Y comprises the

15 polypeptide segment E, or is absent; wherein Z comprises the polypeptide segment G or is absent; and wherein X comprise a polypeptide segment selected from the group consisting of C/D HKL, C/D H, C/D HL, C/D D, C/D' HL, C/D' HKL, C/D' H, C/D' D, C/D C/D' HKL, C/D C/D' H, C/D C/D' HL, C/D C/D' D, C/d D'H, C/D D' HL, C/D D' HKL, C/D' D' H, C/D' D' HL, C/D' D' HKL, C/D C/D' D' H, C/D C/D' D' HL and C/D C/D' D' HKL, and preferably that either

- a) at least one of F, Y, B, A, Z, C or X is of bovine origin; or
 - b) Y comprises the polypeptide segment E; or
 - c) X comprises the polypeptide segments C/D HKL, C/D D, C/D' HKL, C/D C/D' HKL, C/D C/D' D, C/D D' H, C/D D' HL, C/D D' HKL, C/D' D' H, C/D' D' HKL, C/D C/D' D'H, C/D C/D' D HL, C/D C/D' D' HKL, C/D'H, C/D C/D' H or C/D C/D' HL.
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Particularly preferred neuregulins also include those polypeptides that include the segments FB polypeptides that include the segments FBA' (i.e. the groups F, B and A' as defined herein including in the drawings); polypeptides that include the segments EBA (i.e. the groups E, B and A as defined herein including in the drawings); polypeptides that include the segments EBA' (i.e. the groups E, B and A' as defined herein including in the drawings); A (i.e. the group A as defined herein

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including in the drawings); polypeptides that include the segments FEBA (i.e. the groups F, E, B and A as defined herein including in the drawings); polypeptides that include the segments FBA' (i.e. the groups F, B and A' as defined herein including in the drawings); and polypeptides that include the segments FEBA' (i.e. the groups F, E, B and A' as defined herein including in the drawings).

Also preferred are nucleic acids that code for the above preferred polypeptides.

A "fragment" or "derivative" of a neuregulin refers to herein 1) a peptide in which one or more amino acid residues are with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) a peptide in which one or more of the amino acid residues includes a substituent group, or (iii) a peptide in which the mature protein is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol). Thus, a fragment or derivative for use in accordance with the methods of the invention includes a proprotein, which can be activated by cleavage of the proprotein portion to produce an active mature polypeptide.

The polypeptide fragments and derivatives of the invention are of a sufficient length to uniquely identify a region of a neuregulin. Neuregulin fragments thus preferably comprise at least 8 amino acids, usually at least about 12 amino acids, more usually at least about 15 amino acids, still more typically at least about 30 amino acids, even more typically at least about 50 or 70 amino acids. Preferred fragments or derivatives for use in the methods of the invention include those that have at least about 70 percent homology (sequence identity) to any of the preferred sequences mentioned above, more preferably about 80 percent or more homology to any of the preferred sequences mentioned above, still more preferably about 85 to 90 percent or more homology to any of the preferred sequences mentioned above. Sequence identity or homology with respect to a neuregulin as referred to herein is the percentage of amino acid sequences of a neuregulin protein or fragment or derivative thereof that are identical with a specified sequence, after introducing any gaps necessary to achieve the maximum percent homology.

The neuregulin fragments and derivatives for use in the methods of the invention preferably exhibit good activity in standard neuroprotective assays such as

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the *in vivo* cerebral ischemia assay of Example 1, which follows. That assay includes the following steps: a) continuous intraventricular infusion of the protein fragment or derivative or vehicle alone to test rats for three days prior to inducing focal ischemic infarcts in right lateral cerebral cortex; and b) twenty-four hours after inducing ischemic infarcts, infarct volume in each test animal is determined by image analysis. Preferably, a protein fragment or derivative of the invention provides at least about a 10% reduction in infarct volume relative to vehicle-treated animals, more preferably about a 20% reduction in infarct volume, still more preferably about a 25% reduction in infarct volume relative to vehicle-treated animals in such an assay. References herein to *in vivo* cerebral ischemia assay are intended to refer to an assay of the above steps a) and b), which are more fully described in Example 1 which follows.

As discussed above, neuregulin nucleic acid fragments and derivatives are also provided for use in the methods of the invention. Those fragments and derivatives typically are of a length sufficient to bind to a sequence of any of the nucleic acid sequences shown in Figures 1-15 of the drawings, including SEQ ID NOS:1, 3, 6, 7, 10, 11, 13, 16, 17, 20, 21, 24, 25, 28, 29, 31, 32, 34, 36, 37, 40, 42 and 43 under the following moderately stringent conditions (referred to herein as "normal stringency" conditions): use of a hybridization buffer comprising 20% formamide in 0.8M saline/0.08M sodium citrate (SSC) buffer at a temperature of 37°C and remaining bound when subject to washing once with that SSC buffer at 37°C.

Preferred neuregulin nucleic acid fragments and derivatives of the invention will bind to a sequence of any of the nucleic acid sequences shown in Figures 1-15 of the drawings, including SEQ ID NOS:1, 3, 6, 7, 10, 11, 13, 16, 17, 20, 21, 24, 25, 28, 29, 31, 32, 34, 36, 37, 40, 42 and 43 under the following highly stringent conditions (referred to herein as "high stringency" conditions): use of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M sodium citrate (SSC) buffer at a temperature of 42°C and remaining bound when subject to washing twice with that SSC buffer at 42°C.

The neuregulin nucleic acid fragments and derivatives preferably should comprise at least 20 base pairs, more preferably at least about 50 base pairs, and still more preferably a nucleic acid fragment or derivative of the invention comprises at least about 100, 200, 300 or 400 base pairs. In some preferred embodiments, the

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nucleic acid fragment or derivative is bound to some moiety which permits ready identification such as a radionucleotide, fluorescent or other chemical identifier.

Isolated neuregulin and peptide fragments or derivatives of the invention are preferably produced by recombinant methods, although suitable neuregulins also can be isolated from various sources. See the procedures disclosed U.S. Patent 5,530,109; 5 U.S. Patent 5,367,060; and PCT/US93/07491, incorporated herein by reference. A wide variety of molecular and biochemical methods are available for generating and expressing neuregulin; see e.g. the procedures disclosed in *Molecular Cloning, A Laboratory Manual* (2nd Ed., Sambrook, Fritsch and Maniatis, Cold Spring Harbor), 10 *Current Protocols in Molecular Biology* (Eds. Ausubel, Brent, Kingston, More, Feidman, Smith and Stuhl, Greene Publ. Assoc., Wiley-Interscience, NY, N.Y. 1992) or other procedures that are otherwise known in the art. For example, neuregulin or fragments or derivatives thereof may be obtained by chemical synthesis, or more preferably by expression in bacteria such as *E coli* and eukaryotes such as yeast, 15 baculovirus, or mammalian cell-based expression systems, etc., depending on the size, nature and quantity of neuregulin or fragment or derivative thereof. More particularly, a recombinant DNA molecule comprising a vector and a DNA segment encoding neuregulin, or a fragment or derivative thereof, can be constructed. Suitable vectors include e.g. baculovirus-derived vectors for expression in insect cells (see 20 Pennock et al., *Mol. Cell. Biol.*, 4:399-406 (1984)), T7-based expression vector for expression in bacteria (see Rosenberg et al., *Gene*, 56:125-135 (1987)) and the pMSXND expression vector for expression in mammalian cells (Lee and Nathans, *J. Biol. Chem.*, 263:3521-3527 (1988)). The DNA segment can be present in the vector operably linked to regulatory elements, e.g., a promoter (e.g., polyhedron, T7 or 25 metallothionein (Mt-I) promoters), or a leader sequence to provide for secretory expression of the polypeptide. The recombinant DNA molecule containing the DNA coding for a neuregulin or a fragment or derivative thereof can be introduced into appropriate host cells by known methods. Suitable host cells include e.g. prokaryotes such as *E. coli*, *Bacillus subtilis*, etc., and eukaryote such as animal cells and yeast 30 strains, e.g., *S. cerevisiae*. Mammalian cells may be preferred such as J558, NSO, SP2-O or CHO. In general, conventional culturing conditions can be employed. See Sambrook, *supra*. Stable transformed or transfected cell lines can then be selected.

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The expressed neuregulin or fragment or derivative thereof then can be isolated and purified by known methods. Typically the culture medium is centrifuged and the supernatant purified by affinity or immunoaffinity chromatography, e.g. Protein-A or Protein-G affinity chromatography or an immunoaffinity protocol comprising use of
5 monoclonal antibodies that bind neuregulins.

Neuregulin nucleic acids used in the methods of the invention are typically isolated, meaning the nucleic acids comprise a sequence joined to a nucleotide other than that which it is joined to on a natural chromosome and usually constitute at least about 0.5%, preferably at least about 2%, and more preferably at least about 5% by
10 weight of total nucleic acid present in a given fraction. A partially pure nucleic acid constitutes at least about 10%, preferably at least about 30%, and more preferably at least about 60% by weight of total nucleic acid present in a given fraction. A pure nucleic acid constitutes at least about 80%, preferably at least about 90%, and more preferably at least about 95% by weight of total nucleic acid present in a given
15 fraction.

As discussed above, the present invention includes methods for treating and preventing certain neurological-related injuries and disorders, comprising the administration of an effective amount of a neuregulin or fragment or derivative thereof, or nucleic acid encoding same, to a subject including a mammal, particularly
20 a human, in need of such treatment.

In particular, the invention provides methods for treatment and/or prophylaxis of nerve cell death (degeneration) resulting from hypoxia, hypoglycemia, brain or spinal cord ischemia, brain or spinal cord trauma, stroke, heart attack or drowning. Typical candidates for treatment include e.g. heart attack, stroke and/or persons
25 suffering from cardiac arrest neurological deficits, brain or spinal cord injury patients, patients undergoing major surgery such as heart surgery where brain ischemia is a potential complication and patients such as divers suffering from decompression sickness due to gas emboli in the blood stream. Candidates for treatment also will include those patients undergoing a surgical procedure involving extra-corporal
30 circulation such as e.g. a bypass procedure.

The invention also provides methods for treatment which comprise administration of a neuregulin or fragment or derivative thereof, or nucleic acid

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encoding same, to a patient that is undergoing surgery or other procedure where brain or spinal cord ischemia is a potential risk. For example, carotid endarterectomy is a surgical procedure employed to correct atherosclerosis of the carotid arteries. Major risks associated with the procedure include intraoperative embolization and the danger of hypertension in the brain following increased cerebral blood flow, which may result in aneurysm or hemorrhage. Thus, an effective amount of a neuregulin or fragment or derivative thereof, or nucleic acid encoding same, could be administered pre-operatively or peri-operatively to reduce such risks associated with carotid endarterectomy, or other post-surgical neurological deficits.

10 The invention also is effective to promote and enhance recovery from acute nerve cell death and neurological conditions. Thus, for example, a neuregulin or fragment or derivative thereof, or nucleic acid encoding same, could be administered to promote repair, remodeling or reprogramming to a patient that has suffered from stroke or other neuronal injury, suitably for an extended period as discussed above. A
15 therapeutic agent of the invention also could be administered post-operatively to promote recovery from any neurological deficits that may have occurred to a patient that has undergone surgery.

 The invention further includes methods for prophylaxis against neurological deficits resulting from e.g. coronary artery bypass graft surgery and aortic valve
20 replacement surgery, or other procedure involving extra-corporal circulation. Those methods will comprise administering to a patient undergoing such surgical procedures an effective amount of a neuregulin or fragment or derivative thereof, or nucleic acid encoding same, typically either pre-operatively or peri-operatively.

 The invention also provides methods for prophylaxis and treatment against
25 neurological injury for patients undergoing myocardial infarction, a procedure that can result in ischemic insult to the patient. Such methods will comprise administering to a patient undergoing such surgical procedure an effective amount of a neuregulin or fragment or derivative thereof, or nucleic acid encoding same, typically either pre-operatively or peri-operatively.

30 Also provided are methods for treating or preventing neuropathic pain such as may be experienced by cancer patients, persons having diabetes, amputees and other persons who may experience neuropathic pain. These methods for treatment comprise

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administration of an effective amount of a neuregulin or fragment or derivative thereof, or nucleic acid encoding same, to a patient in need of such treatment.

The invention also provides methods for treatment and prophylaxis against retinal ischemia or degeneration and resulting visual loss. For example, a neuregulin or fragment or derivative thereof, can be administered parenterally or by other
5 procedure as described herein to a subject suffering from or susceptible to ischemic insult that may adversely affect retinal function, e.g., significantly elevated intraocular pressures, diseases such as retinal artery or vein occlusion, diabetes or other ischemic ocular-related diseases. Post-ischemic administration also may limit retinal damage.

10 The invention also includes methods for treating and prophylaxis against decreased blood flow or nutrient supply to retinal tissue or optic nerve, or treatment or prophylaxis against retinal trauma or optic nerve injury. Subjects for treatment according to such therapeutic methods of the invention may be suffering or susceptible to retinal ischemia that is associated with atherosclerosis, venous capillary
15 insufficiency, obstructive arterial or venous retinopathies, senile macular degeneration, cystoid macular edema or glaucoma, or the retinal ischemia may be associated with a tumor or injury to the mammal. Intravitreal injection also may be a preferred administration route to provide more direct treatment to the ischemic retina.

The invention further provides a method of treating Korsakoff's disease, a
20 chronic alcoholism-induced condition, comprising administering to a subject including a mammal, particularly a human, an effective amount of a neuregulin or fragment or derivative thereof, in an amount effective to treat the disease.

Compounds of the invention are anticipated to have utility for the attenuation of cell loss, hemorrhages and/or amino acid changes associated with Korsakoff's disease.

25 The invention further includes methods for treating a person suffering from or susceptible to epilepsy, emesis, narcotic withdrawal symptoms and age-dependent dementia, comprising administering to a subject including a mammal, particularly a human, an effective amount of a neuregulin or fragment or derivative thereof, in an amount effective to treat the condition.

30 It will be appreciated that in some instances a neuregulin or a fragment or derivative thereof will be preferably administered to a subject rather than a neuregulin nucleic acid, particularly where a patient is suffering from or susceptible to an acute

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neurological injury that demands immediate therapy. For example, administration of a neuregulin polypeptide may be preferred to a patient suffering from stroke, heart attack, traumatic brain injury and the like where it is desired to deliver the active therapeutic as quickly as possible.

5 In the therapeutic methods of the invention, neuregulin peptides and nucleic acids may be suitably administered to a subject such as a mammal, particularly a human, by any of a number of routes including parenteral (including subcutaneous, intramuscular, intravenous and intradermal), oral, rectal, nasal, vaginal and optical (including buccal and sublingual) administration. A neuregulin protein or nucleic acid
10 or fragment or derivative thereof may be administered to a subject alone or as part of a pharmaceutical composition, comprising the peptide or nucleic acid together with one or more acceptable carriers and optionally other therapeutic ingredients. The carriers should be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

15 Nucleic acids encoding a neuregulin or a neuregulin fragment or derivative can be administered to a patient by generally known gene therapy procedures. See, for example, WO 90/11092 and WO 93/00051. Thus, for instance, the nucleic acids may be introduced into target cells by any method which will result in the uptake and expression of the nucleic acid by the target cells. These methods can include vectors,
20 liposomes, naked DNA, adjuvant-assisted DNA, catheters, etc. Preferably, the administered nucleic acid codes for an appropriate secretory sequence to promote expression upon administration. Suitable vectors for administering a nucleic acid in accordance with the invention include chemical conjugates such as described in WO 93/04701, which has targeting moiety (e.g. a ligand to a cellular surface receptor), and
25 a nucleic acid binding moiety (e.g. polylysine), viral vector (e.g. a DNA or RNA viral vector), fusion proteins such as described in PCT/US 95/02140 (WO 95/22618) which is a fusion protein containing a target moiety (e.g. an antibody specific for a target cell) and a nucleic acid binding moiety (e.g. a protamine), plasmids, phage, etc. The vectors can be chromosomal, non-chromosomal or synthetic.

30 Preferred vectors include viral vectors, fusion proteins and chemical conjugates. Retroviral vectors include moloney murine leukemia viruses. DNA viral vectors are preferred. These vectors include pox vectors such as orthopox or avipox

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vectors, herpes virus vectors such as a herpes simplex I virus (HSV) vector [A.I. Geller et al., *J. Neurochem*, 64:487 (1995); F. Lim et al., in *DNA Cloning: Mammalian Systems*, D. Glover, Ed. (Oxford Univ. Press, Oxford England) (1995); A.I. Geller et al., *Proc Natl. Acad. Sci. U.S.A.*:90 7603 (1993); A.I. Geller et al., *Proc Natl. Acad. Sci USA*, 87:1149 (1990)], Adenovirus Vectors [LeGal LaSalle et al., *Science*, 259:988 (1993); Davidson, et al., *Nat. Genet.*, 3:219 (1993); Yang et al., *J. Virol.*, 69:2004 (1995)] and Adeno-associated Virus Vectors [Kaplitt, M.G., et al., *Nat. Genet.*, 8:148 (1994)].

Pox viral vectors introduce the gene into the cell cytoplasm. Avipox virus vectors result in only a short-term expression of the nucleic acid. Adenovirus vectors, adeno-associated virus vectors and herpes simplex virus (HSV) vectors are preferred for introducing the nucleic acid into neural cells. The adenovirus vector results in a shorter term expression (about 2 months) than adeno-associated virus (about 4 months), which in turn is shorter than HSV vectors. The particular vector chosen will depend upon the target cell and the specific condition being treated. The introduction can be by standard techniques, e.g. infection, transfection, transduction or transformation. Examples of modes of gene transfer include e.g., naked DNA, $\text{Ca}_3(\text{PO}_4)_2$ precipitation, DEAE dextran, electroporation, protoplast fusion, lipofecton, cell microinjection, and viral vectors.

A vector can be employed to target essentially any desired target cell. For example, stereotaxic injection can be used to direct the vectors (e.g. adenovirus, HSV) to a desired location. Additionally, the particles can be delivered by intracerebroventricular (icv) infusion using a minipump infusion system, such as a SynchroMed Infusion System. A method based on bulk flow, termed convection, has also proven effective at delivering large molecules to extended areas of the brain and may be useful in delivering the vector to the target cell (Bobo et al., *Proc. Natl. Acad. Sci. USA*, 91:2076-2080 (1994); Morrison et al., *Am. J. Physiol.*, 266:292-305 (1994)). Other methods that can be used include catheters, intravenous, parenteral, intraperitoneal and subcutaneous injection, and oral or other known routes of administration.

Parenteral formulations for administration of a neuregulin or a fragment or derivative thereof may be in the form of liquid solutions or suspensions; for oral

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administration, formulations may be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols.

Methods well known in the art for making formulations are found in, for example, "Remington's Pharmaceutical Sciences". Formulations for parenteral administration may, for example, contain as excipients sterile water or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated naphthalenes, biocompatible, biodegradable lactide polymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the present factors. Other potentially useful parenteral delivery systems for a neuregulin or fragments or derivatives thereof include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain as excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel to be applied intranasally. Formulations for parenteral administration may also include glycocholate for buccal administration, methoxysalicylate for rectal administration, or citric acid for vaginal administration.

The concentration of a neuregulin or a fragment or derivative thereof, or nucleic acid encoding such polypeptides, administered to a particular subject will vary depending upon a number of issues, including the condition being treated, the mode and site of administration, the age, weight sex and general health of the subject, and other such factors that are recognized by those skilled in the art. Optimal administration rates for a given protocol of administration can be readily determined by those skilled in the art.

All documents mentioned herein are incorporated herein by reference in their entirety. The invention is further illustrated by the following non-limiting Examples. Example 1 -- In vivo neuroprotection assay

Neuregulins and neuregulin fragments and derivatives can be assessed for neuroprotective efficacy pursuant to the following assay.

Mature male Long-Evans rats (Charles River, 250-350g) are allowed food and water *ad libitum*. Animals are anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and placed in a stereotaxic head holder (David Kopf Instruments, Tujunga, CA). The

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dorsal surface of the skull is exposed by midline incision, and a small burr hole (2 mm diameter) is drilled over the right lateral ventricle, 1.6 mm lateral and 0.9 mm posterior to bregma. A stainless steel cannula (I.D. 0.020", O.D. 0.028", 2 cm long) is then inserted stereotaxically into the ventricle to a depth of 4.4 mm beneath the surface of the skull. The tubing is suitably bent at a 90° angle 1-1.6 cm from its tip and connected to polyethylene tubing (I.D. 0.76 mm, O.D. 1.22 mm, 10 cm long) that is connected (by glue) to a mini-osmotic pump (Alzet 1007D, 100 µl fill volume, pump rate = 0.5 µl/hr; Alza Corp., Palo Alto, CA) implanted subcutaneously in the back. The cannula can be suitably fixed to the skull by orthodontic resin (L.D. Culk Co., Milford, DE) bonded to two small machine screws (1/8" stainless steel slotted) inserted in the skull. The pump, tubing, and cannula are primed before insertion with infusate solutions; a 3-0 nylon suture is inserted into the cannula during implantation to prevent obstruction by brain tissue. The wound is closed with 3-0 silk suture and cefazolin (10 mg, i.m.) is administered. After surgery animals are suitably kept in individual cages and fed soft food.

Pumps are filled with vehicle alone (containing 127 mM NaCl, 2.6 mM KCl, 1.2 mM CaCl₂, 0.9 mM MgCl₂, 4.14 mM HEPES, 3 mM glycerin, 0.001% bovine serum albumin [BSA], and 0.01% fast green), or vehicle neuregulin or fragment or derivative thereof (100 µgm/ml). Heparin can be suitably used at relatively low doses, e.g. about 0.8 units/kg/day which is approximately 250-500 times less than a standard anticoagulant dose.

Three days after cannula implantation, animals are reanesthetized with 2% halothane and given atropine (0.15 mg/kg, i.p.). Animals are then intubated and connected to a ventilator (SAR-830; CWE Inc., Ardmore, PA) delivering 1% halothane/70% nitrous oxide in oxygen. The right femoral artery and vein are cannulated for monitoring of mean arterial blood pressure (MABP; Gould RS3200 Blood Pressure Monitor, Gould Inc., Valley View, OH), and blood sampling. Animals are then paralyzed with pancuronium bromide (0.5 mg/kg, i.v.). Arterial blood gasses (Corning 178 Blood Gas Analyzer, Ciba Corning Diagnostic Corp., Medford, MA), blood glucose (Accu-Check Blood Glucose Analyzer, Boehringer Mannheim, Indianapolis, IN), and hematocrit are measured at least twice during surgery and the immediate post-operative period. The stroke volume and rate of the

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ventilator are adjusted to maintain PaO₂ between 100-200 mm Hg and PaCO₂ between 30-40 mm Hg. Core body temperature may be monitored by rectal thermocouple (e.g. Model 73ATA, Yellow Springs Instrument Co., Yellow Springs, OH) and maintained between 36-37°C with a homeothermic blanket control unit (Harvard Bioscience, South Natick, MA).

Focal ischemic infarcts are made in the right lateral cerebral cortex in the territory of the middle cerebral artery (MCA) by the method of Chen, et al., *Stroke*, 17:738-743 (1986). Both common carotid arteries are exposed by midline anterior cervical incision. The animal is placed in a lateral position and a 1 cm skin incision is then made at the midpoint between the right lateral canthus and the anterior pinna. The temporal muscle is retracted, and a small (3 mm diameter) craniectomy is made at the junction of the zygoma and squamosal bone using a dental drill cooled with saline. Using a dissecting microscope, the dura can be opened with fine forceps, and the right MCA can be ligated with two 10-0 monofilament nylon ties just above the rhinal fissure and transected between the ties. Both common carotid arteries then can be occluded by microaneurysm clips for 45 minutes. After removal of the clips, return of flow is visualized in the arteries. Anesthesia is maintained for 15 minutes, and animals are returned to individual cages and fed soft food after surgery.

Twenty four hours after cerebral infarction, animals are again weighed, and then sacrificed by rapid decapitation. Brains are removed, inspected visually for the anatomy of the middle cerebral artery as well as for signs of hemorrhage or infection, immersed in cold saline for 10 minutes, and sectioned into six standard coronal slices (each 2 mm thick) using a rodent brain matrix slicer (Systems, Warren, MI). Brains are also examined visually for the presence of dye (fast green) in the cerebral ventricles. Slices are placed in the vital dye 2,3,5-triphenyl tetrazolium chloride (TTC, 2%; Chemical Dynamics Co., NH) at 37°C in the dark for 30 minutes, followed by 10% formalin at room temperature overnight. The outline of right and left cerebral hemispheres as well as that of infarcted tissue, clearly visualizable by lack of TTC staining (Chen et al., *Stroke*, 17:738-743 (1986)), is outlined on the posterior surface of each slice using an image analyzer (MTI videocamera and Sony video monitor connected to a Bioquant IV Image Analysis System run on an EVEREX computer). Infarct volume is calculated as the sum of infarcted area per slice multiplied by slice

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thickness. Both the surgeon and image analyzer operator are blinded to the treatment given each animal.

Volumes of infarcts among vehicle vs. neuregulin-treated animals can be compared by unpaired, two-tailed t-tests for each experiment, and by two-way analysis of variance (ANOVA; Exp. X Treatment) for combined data. A subsequent slice-by-slice analysis of infarct area among pooled neuregulin- vs. vehicle-treated animals is suitably done by repeated measures two-way ANOVA (Treatment X Slice).

Other anatomical and physiological measurements are compared among GDF-1- vs. vehicle-treated animals by unpaired, two-tailed t-tests using the Bonferroni correction for multiple pairwise comparisons.

Example 2 -- In vivo behavioral assays

For behavioral outcome studies, such as to assess recovery, repair and remodeling promoted by administration of a neuregulin or fragment or derivative thereof, or nucleic acid encoding same, a number of assays can be employed such as those described in G. Sinson et al., *J Neurochem*, 65(5):2209-2214 (1995); T.K. McIntosh et al., *Neuroscience*, 28:233-244 (1989); and T.K. McIntosh et al., *J Neurotrauma*, 10:373-384 (1993).

Briefly, one suitable behavioral assay as described in G. Sinson et al., *supra*, entails that test animals (male Sprague-Dawley rats) receive preinjury training in a Morris Water Maze, a circular tank 1 m in diameter that is filled with 18°C water. The water surface is made opaque with a covering of Styrofoam pieces. During training of the animals a submerged platform is present in the maze. Each test animal undergoes 20 training trials over a two day period during which they learn to locate the platform using external visual cues. Immediately following the last training trial, animals are anesthetized and subjected to a lateral (parasagittal) fluid-percussion (FP) brain injury. Briefly, a 5-mm craniectomy is performed over the left parietal cortex, midway between lamda and bregma. A hollow Leur-loc fitting is cemented to the craniectomy site. The injury is delivered after attaching the FP device. The injury should be of moderate severity (2.1-2.3 atm). After injury, the Leur-loc is removed, and the skin is sutured. Normothermia is maintained with warming pads until the animals begin to ambulate.

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At 72 hours, 1 week or 2 weeks after injury, animals are assessed for their ability to remember the learned task of locating the platform in the MWM. For this evaluation the platform is removed from the maze, and the animal's swimming pattern is suitably recorded with a computerized video system for 1 minute. The maze is
5 separated in zones that are weighed according to the proximity to the platform's location. A memory score is generated by multiplying the weighted numbers by the time the animal spends in each zone and then adding the products.

Animals surviving for 1 or 2 weeks also can undergo evaluation of neurologic motor function. Briefly, one suitable assay provides that animals are scored from 0
10 (severely impaired) to 4 (normal) for each of the following: (1) left and (2) right forelimb during suspension by the tail; (3) left and (4) right hindlimb flexion when the forelimbs remain on a surface and the hindlimbs are lifted up and back by the tail; the ability to resist lateral pulsion to the (5) left and (6) right; and the ability to stand on an inclined plane in the (7) left, (8) right, and (9) vertical positions. Scores are
15 combined for each of the tests (1) through (9). The observer for the tests should be blinded to the animal's previous treatment.

The invention has been described in detail with reference to preferred embodiments thereof. However, it will be appreciated that those skilled in the art, upon consideration of this disclosure, may make modifications and improvements
20 within the spirit and scope of the invention.

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What is claimed is:

1. A method of treating a mammal suffering from or susceptible to stroke, brain or spinal cord injury or ischemia, or heart attack, comprising administering to the mammal a therapeutically effective amount of a neuregulin, or fragment or derivative of a neuregulin, or a nucleic acid encoding a neuregulin or a fragment or derivative of a neuregulin.

2. A method of treating a mammal suffering from or susceptible to optic nerve injury or retinal injury or ischemia, comprising administering to the mammal a therapeutically effective amount of a neuregulin, or fragment or derivative of a neuregulin, or a nucleic acid encoding a neuregulin or a fragment or derivative of a neuregulin.

3. A method of treating a mammal suffering from or susceptible to effects of post-surgical neurological deficits, hypoxia or hypoglycemia, comprising administering to the mammal a therapeutically effective amount of a neuregulin, or fragment or derivative of a neuregulin, or a nucleic acid encoding a neuregulin or a fragment or derivative of a neuregulin.

4. A method of treating a mammal suffering from or susceptible to epilepsy, Parkinson's disease, Huntington's disease, Amyotrophic Lateral Sclerosis, Alzheimer's disease, Down's Syndrome, Korsakoff's disease, or age-dependent dementia, comprising administering to the mammal a therapeutically effective amount of a neuregulin, or fragment or derivative of a neuregulin, or a nucleic acid encoding a neuregulin or a fragment or derivative of a neuregulin.

5. The method of claim 1 wherein the neuregulin, or fragment or derivative of a neuregulin, or a nucleic acid encoding a neuregulin or a fragment or derivative of a neuregulin is administered after the subject has suffered a stroke, brain or spinal cord injury or ischemia, or heart attack.

6. The method of claim 5 wherein the neuregulin, or fragment or derivative of a neuregulin, or a nucleic acid encoding a neuregulin or a fragment or derivative of a neuregulin is administered to the subject for at least about two weeks after the subject has suffered a stroke, brain or spinal cord injury or ischemia, or heart attack.

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7. A method of any one of claims 1-6 wherein a neuregulin or a fragment or derivative thereof is administered to the mammal.

8. A method of claim 7 wherein the neuregulin or fragment or derivative thereof comprises an amino acid sequence of the following formula:

WYBAZCX

wherein WYBAZCX is composed of amino acid sequences that include one or more sequences shown in FIGS. 1 through 15 (which includes SEQ ID NOS:2, 4, 5, 8, 9, 12, 14, 15, 18, 19, 22, 23, 26, 27, 30, 33, 35, 38, 39, 41, 44, 45 and 48), wherein W comprises the polypeptide segment F, or is absent; wherein Y comprises the polypeptide segment E, or is absent; wherein Z comprises the polypeptide segment G or is absent; and wherein X comprise a polypeptide segment selected from the group consisting of C/D HKL, C/D H, C/D HL, C/D D, C/D' HL, C/D' HKL, C/D' H, C/D' D, C/D C/D' HKL, C/D C/D' H, C/D C/D' HL, C/D C/D' D, C/d D'H, C/D D' HL, C/D D' HKL, C/D' D' H, C/D' D' HL, C/D' D' HKL, C/D C/D' D' H, C/D C/D' D' HL and C/D C/D' D' HKL, and preferably that either

- a) at least one of F, Y, B, A, Z, C or X is of bovine origin; or
- b) Y comprises the polypeptide segment E; or
- c) X comprises the polypeptide segments C/D HKL, C/D D, C/D' HKL, C/D C/D' HKL, C/D C/D' D, C/D D' H, C/D D' HL, C/D D' HKL, C/D' D' H, C/D' D' HKL, C/D C/D' D'H, C/D C/D' D HL, C/D C/D' D' HKL, C/D'H, C/D C/D' H or C/D C/D' HL.

9. The method of claim 7 wherein the neuregulin or fragment or derivative thereof a) has at least one of F, Y, B, A, Z, C or X is of bovine origin; or b) Y comprises the polypeptide segment E; or c) X comprises the polypeptide segments C/D HKL, C/D D, C/D' HKL, C/D C/D' HKL, C/D C/D' D, C/D D H, C/D D' HL, C/D D' HKL, C/D C/D' D' H, C/D C/D' D HL, C/D C/D' D' HKL, C/D'H, C/D C/D' H or C/D C/D' HL.

10. The method of claim 7 wherein the neuregulin or fragment or derivative thereof comprises FBA polypeptide segments, FEBA polypeptides segments, EBA polypeptide segments, EBA' polypeptide segments or FEBA' polypeptide segments.

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11. A method of claim 7 wherein the neuregulin is encoded by a nucleic acid that comprises one of SEQ ID NOS:49, 51 and 53.
12. A method of claim 7 wherein the neuregulin or fragment or derivative thereof is encoded by a nucleic acid that comprises a sequence that has at least about 70% sequence identity to one of SEQ ID NOS:49, 51 and 53.
13. A method of claim 7 wherein the neuregulin or fragment or derivative thereof is encoded by a sequence that hybridizes to one of SEQ ID NOS:49, 51 or 53 under normal stringency conditions.
14. A method of claim 7 wherein the neuregulin or fragment or derivative thereof is encoded by a sequence that hybridizes to one of SEQ ID NOS:49, 51 or 53 under high stringency conditions.
15. A method of claim 7 wherein the neuregulin or fragment or derivative has at least about 70% sequence identity to SEQ ID NOS:50, 52 or 54.
16. A method of claim 7 wherein the neuregulin or fragment or derivative thereof is encoded by a nucleic acid that comprises a sequence that has at least about 70% sequence identity to one of SEQ ID NO:20 (Figure 7); SEQ ID NO:21 (Figure 7); SEQ ID NO:24 (Figure 8); SEQ ID NO:25 (Figure 8); SEQ ID NO:28 (Figure 9); or SEQ ID NO:29 (Figure 9).
17. A method of claim 7 wherein the neuregulin or fragment or derivative thereof is encoded by a sequence that hybridizes to one of SEQ ID NO:20 (Figure 7); SEQ ID NO:21 (Figure 7); SEQ ID NO:24 (Figure 8); SEQ ID NO:25 (Figure 8); SEQ ID NO:28 (Figure 9); or SEQ ID NO:29 (Figure 9) under normal stringency conditions.
18. A method of claim 7 wherein the neuregulin or fragment or derivative comprises a sequence that has at least about 70% sequence identity to any of the peptide sequences shown in Figures 7, 8 or 9 of the drawings.
19. A method of claim 7 where the neuregulin or fragment or derivative comprises a sequence that has at least about 80 percent homology to any of the peptide sequences shown in Figures 7, 8 or 9.
20. A method of claim 7 where the neuregulin or fragment or derivative comprises a sequence that has at least about 90 percent homology to any of the peptide sequences shown in Figures 7, 8 or 9.

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21. A method of claim 7 wherein the neuregulin or fragment or derivative comprises a sequence that has at least about 95 percent homology to any of the peptide sequences shown in Figures 7, 8 or 9.

22. A method of claim 7 wherein the neuregulin or fragment or derivative comprises a sequence that is shown in Figures 7, 8 or 9.

23. A method of any one of claims 1-6 wherein a nucleic acid encoding a neuregulin or a fragment or derivative thereof is administered to the mammal.

24. A method of claim 23 wherein the nucleic acid is SEQ ID NO:49, 51 or 53, or the complement thereof.

25. A method of claim 23 wherein the nucleic or fragment or derivative thereof encodes a neuregulin or neuregulin fragment or derivative that comprises an amino acid sequence of the following formula:

WYBAZCX

wherein WYBAZCX is composed of amino acid sequences that include one or more sequences shown in FIGS. 1 through 15 (which includes SEQ ID NOS:2, 4, 5, 8, 9, 12, 14, 15, 18, 19, 22, 23, 26, 27, 30, 33, 35, 38, 39, 41, 44, 45 and 48), wherein W comprises the polypeptide segment F, or is absent, wherein Y comprises the polypeptide segment E, or is absent; wherein Z comprises the polypeptide segment G or is absent; and wherein X comprise a polypeptide segment selected from the group consisting of C/D HKL, C/D H, C/D HL, C/D D, C/D' HL, C/D' HKL, C/D' H, C/D' D, C/D C/D' HKL, C/D C/D' H, C/D C/D' HL, C/D C/D' D, C/D D' HL, C/D D' HKL, C/D' D' H, C/D' D' HL, C/D' D' HKL, C/D C/D' D' H, C/D C/D' D' HL and C/D C/D' D' HKL.

26. The method of claim 25 wherein the neuregulin or neuregulin fragment or derivative a) has at least one of F, Y, B, A, Z, C or X is of bovine origin; or b) Y comprises the polypeptide segment E; or c) X comprises the polypeptide segments C/D HKL, C/D D, C/D' HKL, C/D C/D' HKL, C/D C/D' D, C/D D H, C/D D' HL, C/D D' HKL, C/D C/D' D' H, C/D C/D' D HL, C/D C/D' D' HKL, C/D'H, C/D C/D' H or C/D C/D' HL.

27. The method of claim 25 wherein the neuregulin or neuregulin fragment or derivative comprises FBA polypeptide segments, FEBA polypeptides segments,

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EBA polypeptide segments, EBA' polypeptide segments or FEBA' polypeptide segments.

28. A method of claim 23 wherein the nucleic acid comprises a sequence that hybridizes to SEQ ID NO:20 (Figure 7); SEQ ID NO:21 (Figure 7); SEQ ID NO:24 (Figure 8); SEQ ID NO:25 (Figure 8); SEQ ID NO:28 (Figure 9); or SEQ ID NO:29 (Figure 9) under normal stringency conditions.

29. A method of claim 23 wherein the nucleic acid comprises a sequence that hybridizes to SEQ ID NO:20 (Figure 7); SEQ ID NO:21 (Figure 7); SEQ ID NO:24 (Figure 8); SEQ ID NO:25 (Figure 8); SEQ ID NO:28 (Figure 9); or SEQ ID NO:29 (Figure 9) under high stringency conditions.

30. A method of claim 23 wherein the nucleic acid comprises a sequence that has at least about 70 percent homology to any of the nucleic acid sequences shown in Figures 7, 8 or 9.

31. A method of claim 23 wherein the nucleic acid comprises a sequence that has at least about 80 percent homology to any of the nucleic acid sequences shown in Figures 7, 8 or 9.

32. A method of claim 23 wherein the nucleic acid comprises a sequence that has at least about 90 percent homology to any of the nucleic acid sequences shown in Figures 7, 8 or 9.

33. A method of claim 23 wherein the nucleic acid comprises a sequence that has at least about 95 percent homology to any of the nucleic acid sequences shown in Figures 7, 8 or 9.

34. A method of claim 23 wherein the nucleic acid comprises a sequence shown in Figures 7, 8 or 9.

35. A method of any one of claims 1-34 wherein the administered neuregulin fragment or derivative, or the administered nucleic acid encodes a neuregulin fragment or derivative exhibits at least about a 10% reduction in infarct volume in an *in vivo* cerebral ischemia assay.

36. A method of any one of claims 1-35 wherein the mammal is a human.

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FIG. 1A
HUMAN SEGMENT E: (SEQ ID NOS:1-2)

ATG AGA TGG CGA CGC GCC CCG CGC CGC TCC GGG CGT CCC GGC CCC CGG Met Arg Trp Arg Arg Ala Pro Arg Arg Ser Gly Arg Pro Gly Pro Arg 1 5 10 15 48
GCC CAG CGC CCC GGC TCC GCC GCC CGC TCG TCG CCG CCG CTG CCG CTG Ala Gln Arg Pro Gly Ser Ala Ala Arg Ser Ser Pro Pro Leu Pro Leu 20 25 30 96
CTG CCA CTA CTG CTG CTG CTG GGG ACC GCG GCC CTG GCG CCG GGG GCG Leu Pro Leu Leu Leu Leu Gly Thr Ala Ala Leu Ala Pro Gly Ala 35 40 45 144
GCG GCC GGC AAC GAG GCG GCT CCC GCG GGG GCC TCG GTG TGC TAC TCG Ala Ala Gly Asn Glu Ala Ala Pro Ala Gly Ala Ser Val Cys Tyr Ser 50 55 60 192
TCC CCG CCC AGC GTG GGA TCG GTG CAG GAG CTA GCT CAG CGC GCC GCG Ser Pro Pro Ser Val Gly Ser Val Gln Glu Leu Ala Gln Arg Ala Ala 65 70 75 80 240
GTG GTG ATC GAG GGA AAG GTG CAC CCG CAG CGG CGG CAG CAG GGG GCA Val Val Ile Glu Gly Lys Val His Pro Gln Arg Arg Gln Gln Gly Ala 85 90 95 288
CTC GAC AGG AAG GCG GCG GCG GCG GCG GCG GAG GCA GGG GCG TGG GGC Leu Asp Arg Lys Ala Ala Ala Ala Ala Gly Glu Ala Gly Ala Trp Gly 100 105 110 336
GGC GAT CGC GAG CCG CCA GCC GCG GGC CCA CGG GCG CTG GGG CCG CCC Gly Asp Arg Glu Pro Pro Ala Ala Gly Pro Arg Ala Leu Gly Pro Pro 115 120 125 384
GCC GAG GAG CCG CTG CTC GCC GCC AAC GGG ACC GTG CCC TCT TGG CCC Ala Glu Glu Pro Leu Leu Ala Ala Asn Gly Thr Val Pro Ser Trp Pro 130 135 140 432
ACC GCC CCG GTG CCC AGC GCC GGC GAG CCC GGG GAG GAG GCG CCC TAT Thr Ala Pro Val Pro Ser Ala Gly Glu Pro Gly Glu Glu Ala Pro Tyr 145 150 155 160 480
CTG GTG AAG GTG CAC CAG GTG TGG GCG GTG AAA GCC GGG GGC TTG AAG Leu Val Lys Val His Gln Val Trp Ala Val Lys Ala Gly Gly Leu Lys 165 170 175 528
AAG GAC TCG CTG CTC ACC GTG CGC CTG GGG ACC TGG GGC CAC CCC GCC Lys Asp Ser Leu Leu Thr Val Arg Leu Gly Thr Trp Gly His Pro Ala 180 185 190 576

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FIG. 1B

TTC	CCC	TCC	TGC	GGG	AGG	CTC	AAG	GAG	GAC	AGC	AGG	TAC	ATC	TTC	TTC	624
Phe	Pro	Ser	Cys	Gly	Arg	Leu	Lys	Glu	Asp	Ser	Arg	Tyr	Ile	Phe	Phe	
		195					200					205				
ATG	GAG	CCC	GAC	GCC	AAC	AGC	ACC	AGC	CGC	GCG	CCG	GCC	GCC	TTC	CGA	672
Met	Glu	Pro	Asp	Ala	Asn	Ser	Thr	Ser	Arg	Ala	Pro	Ala	Ala	Phe	Arg	
	210					215					220					
GCC	TCT	TTC	CCC	CCT	CTG	GAG	ACG	GGC	CGG	AAC	CTC	AAG	AAG	GAG	GTC	720
Ala	Ser	Phe	Pro	Pro	Leu	Glu	Thr	Gly	Arg	Asn	Leu	Lys	Lys	Glu	Val	
225					230					235					240	
AGC	CGG	GTG	CTG	TGC	AAG	CGG	TGC	G								745
Ser	Arg	Val	Leu	Cys	Lys	Arg	Cys									
				245												

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FIG. 2
SEGMENT E: (SEQ ID NOS:3-4)

CC CAT CAA GTG TGG GCG GCG AAA GCC GGG GGC TTG AAG AAG GAC TCG	47
His Gln Val Trp Ala Ala Lys Ala Gly Gly Leu Lys Lys Asp Ser	
1 5 10 15	
CTG CTC ACC GTG CGC CTG GGC GCC TGG GGC CAC CCC GCC TTC CCC TCC	95
Leu Leu Thr Val Arg Leu Gly Ala Trp Gly His Pro Ala Phe Pro Ser	
20 25 30	
TGC GGG CGC CTC AAG GAG GAC AGC AGG TAC ATC TTC TTC ATG GAG CCC	143
Cys Gly Arg Leu Lys Glu Asp Ser Arg Tyr Ile Phe Phe Met Glu Pro	
35 40 45	
GAG GCC AAC AGC AGC GGC GGG CCC GGC CGC CTT CCG AGC CTC CTT CCC	191
Glu Ala Asn Ser Ser Gly Gly Pro Gly Arg Leu Pro Ser Leu Leu Pro	
50 55 60	
CCC TCT CGA GAC GGG CCG GAA CCT CAA GAA GGA GGT CAG CCG GGT GCT	239
Pro Ser Arg Asp Gly Pro Glu Pro Gln Glu Gly Gly Gln Pro Gly Ala	
65 70 75	
GTG CAA CGG TGC G	252
Val Gln Arg Cys	
80	

FIG. 3
SEGMENT B: (SEQ ID NOS:5-8)

Leu Pro Pro Arg Leu Lys Glu His Lys Ser Gln Glu Ser Val Ala Gly	48
CCT TGC CTC CCC GCT TGA AAG AGA TGA AGA GTC AGG AGT CTG TGG CAG	
CCT TGC CTC CCC GAT TGA AAG AGA TGA AAA GCC AGG AAT CGG CTG CAG	
Q A	
Ser Lys Leu Val Leu Arg Cys Glu Thr Ser Ser Glu Tyr Ser Ser Leu	96
GTT CCA AAC TAG TGC TTC GGT GCG AGA CCA GTT CTG AAT ACT CCT CTC	
GTT CCA AAC TAG TCC TTC GGT GTG AAA CCA GTT CTG AAT ACT CCT CTC	
Lys Phe Lys Trp Phe Lys Asn Gly Ser Glu Leu Ser Arg Lys Asn Lys	144
TCA AGT TCA AGT GGT TCA AGA ATG GGA GTG AAT TAA GCC GAA AGA ACA	
TCA GAT TCA AGT GGT TCA AGA ATG GGA ATG AAT TGA ATC GAA AAA ACA	
R N N	
Pro Gly Asn Ile Lys Ile Gln Lys Arg Pro Gly	178
AAC CAC AAA ACA TCA AGA TAC AGA AAA GGC CGG G	
AAC CAC AAA ATA TCA AGA TAC AAA AAA AGC CAG G	
K	

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FIG 4
SEGMENT A: (SEQ ID NOS:9-12)

	Lys	Ser	Glu	Leu	Arg	Ile	Ser	Lys	Ala	Ser	Leu	Ala	Asp	Ser	Gly		
	G	AAG	TCA	GAA	CTT	CGC	ATT	AGC	AAA	GCG	TCA	CTG	GCT	GAT	TCT	GGA	46
	G	AAG	TCA	GAA	CTT	CGC	ATT	AAC	AAA	GCA	TCA	CTG	GCT	GAT	TCT	GGA	
																	N
	Glu	Tyr	Met	Cys	Lys	Val	Ile	Ser	Lys	Leu	Gly	Asn	Asp	Ser	Ala	Ser	
	GAA	TAT	ATG	TGC	AAA	GTG	ATC	AGC	AAA	CTA	GGA	AAT	GAC	AGT	GCC	TCT	94
	GAG	TAT	ATG	TGC	AAA	GTG	ATC	AGC	AAA	TTA	GGA	AAT	GAC	AGT	GCC	TCT	
	Ala	Asn	Ile	Thr	Ile	Val	Glu	Ser	Asn	Ala							
	GCC	AAC	ATC	ACC	ATT	GTG	GAG	TCA	AAC	G							122
	GCC	AAT	ATC	ACC	ATC	GTG	GAA	TCA	AAC	G							

FIG.5
SEGMENT A': (SEQ ID NOS:13-14)

	TCTAAACTA CAGAGACTGT ATTTTCATGA TCATCATAGT TCTGTGAAAT ATACTTAAAC	60
	CGCTTTGGTC CTGATCTTGT AGG AAG TCA GAA CTT CGC ATT AGC AAA GCG	110
	<div style="display: inline-block; width: 40%;">Lys Ser Glu Leu Arg</div> <div style="display: inline-block; width: 40%;">Ile Ser Lys Ala</div>	
	<div style="display: inline-block; width: 40%;">1</div> <div style="display: inline-block; width: 40%;">5</div>	
	TCA CTG GCT GAT TCT GGA GAA TAT ATG TGC AAA GTG ATC AGC AAA CTA	158
	Ser Leu Ala Asp Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys Leu	
	<div style="display: inline-block; width: 25%;">10</div> <div style="display: inline-block; width: 25%;">15</div> <div style="display: inline-block; width: 25%;">20</div> <div style="display: inline-block; width: 25%;">25</div>	
	GGA AAT GAC AGT GCC TCT GCC AAC ATC ACC ATT GTG GAG TCA AAC GGT	206
	Gly Asn Asp Ser Ala Ser Ala Asn Ile Thr Ile Val Glu Ser Asn Gly	
	<div style="display: inline-block; width: 25%;">30</div> <div style="display: inline-block; width: 25%;">35</div> <div style="display: inline-block; width: 25%;">40</div>	
	AAG AGA TGC CTA CTG CGT GCT ATT TCT CAG TCT CTA AGA GGA GTG ATC	254
	Lys Arg Cys Leu Leu Arg Ala Ile Ser Gln Ser Leu Arg Gly Val Ile	
	<div style="display: inline-block; width: 25%;">45</div> <div style="display: inline-block; width: 25%;">50</div> <div style="display: inline-block; width: 25%;">55</div>	
	AAG GTA TGT GGT CAC ACT TGAATCACGC AGGTGTGTGA AATCTCATTG	302
	Lys Val Cys Gly His Thr	
	60	
	TGAACAAATA AAAATCATGA AAGGAAACT CTATGTTTGA AATATCTTAT GGGTCCTCCT	362
	GTAAAGCTCT TCACTCCATA AGGTGAAATA GACCTGAAAT ATATATAGAT TATTT	417

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FIG. 6
SEGMENT G: (SEQ ID NOS:15-18)

Glu	Ile	Thr	Thr	Gly	Met	Pro	Ala	Ser	Thr	Glu	Thr	Ala	Tyr	Val	Ser	
AG	ATC	ACC	ACT	GGC	ATG	CCA	GCC	TCA	ACT	GAG	ACA	GCG	TAT	GTG	TCT	47
AG	ATC	ATC	ACT	GGT	ATG	CCA	GCC	TCA	ACT	GAA	GGA	GCA	TAT	GTG	TCT	
			I								G					
Ser	Glu	Ser	Pro	Ile	Arg	Ile	Ser	Val	Ser	Thr	Glu	Gly	Thr	Asn	Thr	
TCA	GAG	TCT	CCC	ATT	AGA	ATA	TCA	GTA	TCA	ACA	GAA	GGA	ACA	AAT	ACT	95
TCA	GAG	TCT	CCC	ATT	AGA	ATA	TCA	GTA	TCC	ACA	GAA	GGA	GCA	AAT	ACT	
													A			
Ser	Ser	Ser														
TCT	TCA	T														102
TCT	TCA	T														

FIG. 7
SEGMENT C: (SEQ ID NOS:19-22)

Thr	Ser	Thr	Ser	Thr	Ala	Gly	Thr	Ser	His	Leu	Val	Lys	Cys	Ala		
CC	ACA	TCC	ACA	TCT	ACA	GCT	GGG	ACA	AGC	CAT	CTT	GTC	AAG	TGT	GCA	47
CT	ACA	TCT	ACA	TCC	ACC	ACT	GGG	ACA	AGC	CAT	CTT	GTA	AAA	TGT	GCG	
					T											
Glu	Lys	Glu	Lys	Thr	Phe	Cys	Val	Asn	Gly	Gly	Glu	Cys	Phe	Met	Val	
GAG	AAG	GAG	AAA	ACT	TTC	TGT	GTG	AAT	GGA	GGC	GAG	TGC	TTC	ATG	GTG	95
GAG	AAG	GAG	AAA	ACT	TTC	TGT	GTG	AAT	GGA	GGG	GAG	TGC	TTC	ATG	GTG	
Lys	Asp	Leu	Ser	Asn	Pro	Ser	Arg	Tyr	Leu	Cys						
AAA	GAC	CTT	TCA	AAT	CCC	TCA	AGA	TAC	TTG	TGC						128
AAA	GAC	CTT	TCA	AAC	CCC	TCG	AGA	TAC	TTG	TGC						

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FIG. 8
SEGMENT C/D: (SEQ ID NOS:23-26)

Lys	Cys	Gln	Pro	Gly	Phe	Thr	Gly	Ala	Arg	Cys	Thr	Glu	Asn	Val	Pro		48
AAG	TGC	CAA	CCT	GGA	TTC	ACT	GGA	GCG	AGA	TGT	ACT	GAG	AAT	GTG	CCC		
AAG	TGC	CAA	CCT	GGA	TTC	ACT	GGA	GCA	AGA	TGT	ACT	GAG	AAT	GTG	CCC		
Met	Lys	Val	Gln	Thr	Gln	Glu											69
ATG	AAA	GTC	CAA	ACC	CAA	GAA											
ATG	AAA	GTC	CAA	AAC	CAA	GAA											
				N													

FIG. 9
SEGMENT C/D': (SEQ ID NOS:27-29)

Lys	Cys	Pro	Asn	Glu	Phe	Thr	Gly	Asp	Arg	Cys	Gln	Asn	Tyr	Val	Met		48
AAG	TGC	CCA	AAT	GAG	TTT	ACT	GGT	GAT	CGC	TGC	CAA	AAC	TAC	GTA	ATG		
AAG	TGC	CCA	AAT	GAG	TTT	ACT	GGT	GAT	CGC	TGC	CAA	AAC	TAC	GTA	ATG		
Ala	Ser	Phe	Tyr														60
GCC	AGC	TTC	TAC														
GCC	AGC	TTC	TAC														

FIG. 10
SEGMENT D: (SEQ ID NOS:30-32)

Ser	Thr	Ser	Thr	Pro	Phe	Leu	Ser	Leu	Pro	Glu	*						36
AGT	ACG	TCC	ACT	CCC	TTT	CTG	TCT	CTG	CCT	GAA	TAG						
AGT	ACG	TCC	ACT	CCC	TTT	CTG	TCT	CTG	CCT	GAA	TAG						

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FIG. 11
SEGMENT D': (SEQ ID NOS:33-34)

Lys His Leu Gly Ile Glu Phe Met Glu
AAG CAT CTT GGG ATT GAA TTT ATG GAG

27

FIG. 12A
SEGMENT H: (SEQ ID NOS:35-38)

Lys Ala Glu Glu Leu Tyr Gln Lys Arg Val Leu Thr Ile Thr Gly Ile
AAA GCG GAG GAG CTC TAC CAG AAG AGA GTG CTC ACC ATT ACC GGC ATT
||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
AAA GCG GAG GAG CTG TAC CAG AAG AGA GTG CTG ACC ATA ACC GGC ATC

48

Cys	Ile	Ala	Leu	Leu	Val	Val	Gly	Ile	Met	Cys	Val	Val	Val	Tyr	Cys
TGC	ATC	GCG	CTG	CTC	GTG	GTT	GGC	ATC	ATG	TGT	GTG	GTG	GTC	TAC	TGC
TGC	ATC	GCC	CTC	CTT	GTG	GTC	GGC	ATC	ATG	TGT	GTG	GTG	GCC	TAC	TGC

96

Lys	Thr	Lys	Lys	Gln	Arg	Lys	Lys	Leu	His	Asp	Arg	Leu	Arg	Gln	Ser
AAA	ACC	AAG	AAA	CAA	CGG	AAA	AAG	CTT	CAT	GAC	CGG	CTT	CGG	CAG	AGC
AAA	ACC	AAG	AAA	CAG	CGG	AAA	AAG	CTG	CAT	GAC	CGT	CTT	CGG	CAG	AGC

144

Leu	Arg	Ser	Glu	Arg	Asn	Thr	Met	Met	Asn	Val	Ala	Asn	Gly	Pro	His
CTT	CGG	TCT	GAA	AGA	AAC	ACC	ATG	ATG	AAC	GTA	GCC	AAC	GGG	CCC	CAC
CTT	CGG	TCT	GAA	CGA	AAC	AAT	ATG	ATG	AAC	ATT	GCC	AAT	GGG	CCT	CAC
						N				I					

192

His	Pro	Asn	Pro	Pro	Pro	Glu	Asn	Val	Gln	Leu	Val	Asn	Gln	Tyr	Val
CAC	CCC	AAT	CCG	CCC	CCC	GAG	AAC	GTG	CAG	CTG	GTG	AAT	CAA	TAC	GTA
CAT	CCT	AAC	CCA	CCC	CCC	GAG	AAT	GTC	CAG	CTG	GTG	AAT	CAA	TAC	GTA

240

Ser	Lys	Asn	Val	Ile	Ser	Ser	Glu	His	Ile	Val	Glu	Arg	Glu	Ala	Glu
TCT	AAA	AAT	GTC	ATC	TCT	AGC	GAG	CAT	ATT	GTT	GAG	AGA	GAG	GCG	GAG
TCT	AAA	AAC	GTC	ATC	TCC	AGT	GAG	CAT	ATT	GTT	GAG	AGA	GAA	GCA	GAG

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[illegible]

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FIG. 12B

Thr	Val	Thr	Gln	Thr	Pro	Ser	His	Ser	Trp	Ser	Asn	Gly	His	Thr	Glu	
ACT	GTC	ACT	CAG	ACT	CCC	AGT	CAC	AGC	TGG	AGC	AAT	GGA	CAC	ACT	GAA	384
ACT	GTC	ACC	CAG	ACT	CCT	AGC	CAC	AGC	TGG	AGC	AAC	GGA	CAC	ACT	GAA	
Ser	Ile	Ile	Ser	Glu	Ser	His	Ser	Val	Ile	Val	Met	Ser	Ser	Val	Glu	
AGC	ATC	ATT	TCG	GAA	AGC	CAC	TCT	GTC	ATC	GTG	ATG	TCA	TCC	GTA	GAA	432
AGC	ATC	CTT	TCC	GAA	AGC	CAC	TCT	GTA	ATC	GTG	ATG	TCA	TCC	GTA	GAA	
		L														
Asn	Ser	Arg	His	Ser	Ser	Pro	Thr	Gly	Gly	Pro	Arg	Gly	Arg	Leu	Asn	
AAC	AGT	AGG	CAC	AGC	AGC	CCG	ACT	GGG	GGC	CCG	AGA	GGA	CGT	CTC	AAT	480
AAC	AGT	AGG	CAC	AGC	AGC	CCA	ACT	GGG	GGC	CCA	AGA	GGA	CGT	CTT	AAT	
Gly	Leu	Gly	Gly	Pro	Arg	Glu	Cys	Asn	Ser	Phe	Leu	Arg	His	Ala	Arg	
GGC	TTG	GGA	GGC	CCT	CGT	GAA	TGT	AAC	AGC	TTC	CTC	AGG	CAT	GCC	AGA	528
GGC	ACA	GGA	GGC	CCT	CGT	GAA	TGT	AAC	AGC	TTC	CTC	AGG	CAT	GCC	AGA	
	T															
Glu	Thr	Pro	Asp	Ser	Tyr	Arg	Asp	Ser	Pro	His	Ser	Glu	Arg			
GAA	ACC	CCT	GAC	TCC	TAC	CGA	GAC	TCT	CCT	CAT	AGT	GAA	AG			569
GAA	ACC	CCT	GAT	TCC	TAC	CGA	GAC	TCT	CCT	CAT	AGT	GAA	AG			

FIG. 13
SEGMENT K: (SEQ ID NOS:39-40)

A	CAT	AAC	CTT	ATA	GCT	GAG	CTA	AGG	AGA	AAC	AAG	GCC	CAC	AGA	TCC	46
	His	Asn	Leu	Ile	Ala	Glu	Leu	Arg	Arg	Asn	Lys	Ala	His	Arg	Ser	
	1				5					10					15	
AAA	TGC	ATG	CAG	ATC	CAG	CTT	TCC	GCA	ACT	CAT	CTT	AGA	GCT	TCT	TCC	94
Lys	Cys	Met	Gln	Ile	Gln	Leu	Ser	Ala	Thr	His	Leu	Arg	Ala	Ser	Ser	
				20					25					30		
ATT	CCC	CAT	TGG	GCT	TCA	TTC	TCT	AAG	ACC	CCT	TGG	CCT	TTA	GGA	AG	141
Ile	Pro	His	Trp	Ala	Ser	Phe	Ser	Lys	Thr	Pro	Trp	Pro	Leu	Gly	Arg	
			35					40					45			

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FIG. 14A
SEGMENT L: (SEQ ID NOS:41-44)

Tyr Val Ser Ala Met Thr Thr Pro Ala Arg Met Ser Pro Val Asp	
G TAT GTA TCA GCA ATG ACC ACC CCG GCT CGT ATG TCA CCT GTA GAT	46
G TAT GTG TCA GCC ATG ACC ACC CCG GCT CGT ATG TCA CCT GTA GAT	
Phe His Thr Pro Ser Ser Pro Lys Ser Pro Pro Ser Glu Met Ser Pro	
TTC CAC ACG CCA AGC TCC CCC AAG TCA CCC CCT TCG GAA ATG TCC CCG	94
TTC CAC ACG CCA AGC TCC CCC AAA TCG CCC CCT TCG GAA ATG TCT CCA	
Pro Val Ser Ser Thr Thr Val Ser Met Pro Ser Met Ala Val Ser Pro	
CCC GTG TCC AGC ACG ACG GTC TCC ATG CCC TCC ATG GCG GTC AGT CCC	142
CCC GTG TCC AGC ATG ACG GTG TCC ATG CCT TCC ATG GCG GTC AGC CCC	
M	
Phe Val Glu Glu Glu Arg Pro Leu Leu Leu Val Thr Pro Pro Arg Leu	
TTC GTG GAA GAG GAG AGA CCC CTG CTC CTT GTG ACG CCA CCA CGG CTG	190
TTC ATG GAA GAA GAG AGA CCT CTA CTT CTC GTG ACA CCA CCA AGG CTG	
N	
Arg Glu Lys - Tyr Asp His His Ala Gln Gln Phe Asn Ser Phe His	
CGG GAG AAG ... TAT GAC CAC CAC GCC CAG CAA TTC AAC TCG TTC CAC	238
CGG GAG AAG AAG TTT TTT GAC CAT CAC CCT CAG CAG TTC AGC TCC TTC CAC	
K F P	
Cys Asn Pro Ala His Glu Ser Asn Ser Leu Pro Pro Ser Pro Leu Arg	
TGC AAC CCC GCG CAT GAG AGC AAC AGC CTG CCC CCC AGC CCC TTG AGG	286
CAC AAC CCC GCG CAT GAC AGT AAC AGC CTC CCT GCT AGC CCC TTG AGG	
N D A	
Ile Val Glu Asp Glu Glu Tyr Glu Thr Thr Gln Glu Tyr Glu Pro Ala	
ATA GTG GAG GAT GAG GAA TAT GAA ACG ACC CAG GAG TAC GAA CCA GCT	334
ATA GTG GAG GAT GAG GAG TAT GAA ACG ACC CAA GAG TAC GAG CCA GCC	
Gln Glu Pro Val Lys Lys Leu Thr Asn Ser Ser Arg Arg Ala Lys Arg	
CAA GAG CCG GTT AAG AAA CTC ACC AAC AGC AGC CGG CGG GCC AAA AGA	382
CAA GAG CCT GTT AAG AAA CTC GCC AA. .T AGC CGG CGG GCC AAA AGA	
A	

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FIG. 14B

Thr	Lys	Pro	Asn	Gly	His	Ile	Ala	His	Arg	Leu	Glu	Met	Asp	Asn	Asn	
ACC	AAG	CCC	AAT	GGT	CAC	ATT	GCC	CAC	AGG	TTG	GAA	ATG	GAC	AAC	AAC	430
ACC	AAG	CCC	AAT	GGC	CAC	ATT	GCT	AAC	AGA	TTG	GAA	GTG	GAC	AGC	AAC	
								N				V		S		
Thr	Gly	Ala	Asp	Ser	Ser	Asn	Ser	Glu	Ser	Glu	Thr	Glu	Asp	Glu	Arg	
ACA	GGC	GCT	GAC	AGC	AGT	AAC	TCA	GAG	AGC	GAA	ACA	GAG	GAT	GAA	AGA	478
ACA	AGC	TCC	CAG	AGC	AGT	AAC	TCA	GAG	AGT	GAA	ACA	GAA	GAT	GAA	AGA	
	S	S	Q													
Val	Gly	Glu	Asp	Thr	Pro	Phe	Leu	Ala	Ile	Gln	Asn	Pro	Leu	Ala	Ala	
GTA	GGA	GAA	GAT	ACG	CCT	TTC	CTG	GCC	ATA	CAG	AAC	CCC	CTG	GCA	GCC	526
GTA	GGT	GAA	GAT	ACG	CCT	TTC	CTG	GGC	ATA	CAG	AAC	CCC	CTG	GCA	GCC	
								G								
Ser	Leu	Glu	Ala	Ala	Pro	Ala	Phe	Arg	Leu	Val	Asp	Ser	Arg	Thr	Asn	
AGT	CTC	GAG	GCG	GCC	CCT	GCC	TTC	CGC	CTG	GTC	GAC	AGC	AGG	ACT	AAC	574
AGT	CTT	GAG	GCA	ACA	CCT	GCC	TTC	CGC	CTG	GCT	GAC	AGC	AGG	ACT	AAC	
				T						A						
Pro	Thr	Gly	Gly	Phe	Ser	Pro	Gln	Glu	Glu	Leu	Gln	Ala	Arg	Leu	Ser	
CCA	ACA	GGC	GGC	TTC	TCT	CCG	CAG	GAA	GAA	TTG	CAG	GCC	AGG	CTC	TCC	622
CCA	GCA	GGC	CGC	TTC	TCG	ACA	CAG	GAA	GAA	ATC	CAG	GCC	AGG	CTG	TCT	
	A		R			T				I						
Gly	Val	Ile	Ala	Asn	Gln	Asp	Pro	Ile	Ala	Val	*					
GGT	GTA	ATC	GCT	AAC	CAA	GAC	CCT	ATC	GCT	GTC	TAA	AAC	CGA	AAT	ACA	670
AGT	GTA	ATT	GCT	AAC	CAA	GAC	CCT	ATT	GCT	GTA	TAA	AAC	CTA	AAT	AAA	
S																
CCC	ATA	GAT	TCA	CCT	GTA	AAA	CTT	TAT	TTT	ATA	TAA	TAA	AGT	ATT	CCA	718
CAC	ATA	GAT	TCA	CCT	GTA	AAA	CTT	TAT	TTT	ATA	TAA	TAA	AGT	ATT	CCA	
CCT	TAA	ATT	AAA	CAA												733
CCT	TAA	ATT	AAA	CAA												

FIG. 15
SEGMENT F: (SEQ ID NOS:45-48)

SUBSTITUTE SHEET (RULE 26)

FIG. 16A
(SEQ ID NOS:49-50)

SUBSTITUTE SHEET (RULE 26)

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FIG. 16B

AGC	GAG	CAT	ATT	GTT	GAG	AGA	GAG	GCG	GAG	AGC	TCT	TTT	TCC	ACC	AGT	625
Ser	Glu	His	Ile	Val	Glu	Arg	Glu	Ala	Glu	Ser	Ser	Phe	Ser	Thr	Ser	
		195					200					205				
CAC	TAC	ACT	TCG	ACA	GCT	CAT	CAT	TCC	ACT	ACT	GTC	ACT	CAG	ACT	CCC	673
His	Tyr	Thr	Ser	Thr	Ala	His	His	Ser	Thr	Thr	Val	Thr	Gln	Thr	Pro	
	210					215					220					
AGT	CAC	AGC	TGG	AGC	AAT	GGA	CAC	ACT	GAA	AGC	ATC	ATT	TCG	GAA	AGC	721
Ser	His	Ser	Trp	Ser	Asn	Gly	His	Thr	Glu	Ser	Ile	Ile	Ser	Glu	Ser	
225					230				235					240		
CAC	TCT	GTC	ATC	GTG	ATG	TCA	TCC	GTA	GAA	AAC	AGT	AGG	CAC	AGC	AGC	769
His	Ser	Val	Ile	Val	Met	Ser	Ser	Val	Glu	Asn	Ser	Arg	His	Ser	Ser	
				245				250						255		
CCG	ACT	GGG	GGC	CCG	AGA	GGA	CGT	CTC	AAT	GGC	TTG	GGA	GGC	CCT	CGT	817
Pro	Thr	Gly	Gly	Pro	Arg	Gly	Arg	Leu	Asn	Gly	Leu	Gly	Gly	Pro	Arg	
		260					265					270				
GAA	TGT	AAC	AGC	TTC	CTC	AGG	CAT	GCC	AGA	GAA	ACC	CCT	GAC	TCC	TAC	865
Glu	Cys	Asn	Ser	Phe	Leu	Arg	His	Ala	Arg	Glu	Thr	Pro	Asp	Ser	Tyr	
		275					280					285				
CGA	GAC	TCT	CCT	CAT	AGT	GAA	AGA	CAT	AAC	CTT	ATA	GCT	GAG	CTA	AGG	913
Arg	Asp	Ser	Pro	His	Ser	Glu	Arg	His	Asn	Leu	Ile	Ala	Glu	Leu	Arg	
	290					295					300					
AGA	AAC	AAG	GCC	CAC	AGA	TCC	AAA	TGC	ATG	CAG	ATC	CAG	CTT	TCC	GCA	961
Arg	Asn	Lys	Ala	His	Arg	Ser	Lys	Cys	Met	Gln	Ile	Gln	Leu	Ser	Ala	
305					310			315						320		
ACT	CAT	CTT	AGA	GCT	TCT	TCC	ATT	CCC	CAT	TGG	GCT	TCA	TTC	TCT	AAG	1009
Thr	His	Leu	Arg	Ala	Ser	Ser	Ile	Pro	His	Trp	Ala	Ser	Phe	Ser	Lys	
				325				330					335			
ACC	CCT	TGG	CCT	TTA	GGA	AGG	TAT	GTA	TCA	GCA	ATG	ACC	ACC	CCG	GCT	1057
Thr	Pro	Trp	Pro	Leu	Gly	Arg	Tyr	Val	Ser	Ala	Met	Thr	Thr	Pro	Ala	
			340					345					350			
CGT	ATG	TCA	CCT	GTA	GAT	TTC	CAC	ACG	CCA	AGC	TCC	CCC	AAG	TCA	CCC	1105
Arg	Met	Ser	Pro	Val	Asp	Phe	His	Thr	Pro	Ser	Ser	Pro	Lys	Ser	Pro	
	355						360					365				
CCT	TCG	GAA	ATG	TCC	CCG	CCC	GTG	TCC	AGC	ACG	ACG	GTC	TCC	ATG	CCC	1153
Pro	Ser	Glu	Met	Ser	Pro	Pro	Val	Ser	Ser	Thr	Thr	Val	Ser	Met	Pro	
	370					375						380				

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FIG. 16C

TCC	ATG	GCG	GTC	AGT	CCC	TTC	GTG	GAA	GAG	GAG	AGA	CCC	CTG	CTC	CTT	1201
Ser	Met	Ala	Val	Ser	Pro	Phe	Val	Glu	Glu	Glu	Arg	Pro	Leu	Leu	Leu	
385					390					395					400	
GTG	ACG	CCA	CCA	CGG	CTG	CGG	GAG	AAG	TAT	GAC	CAC	CAC	GCC	CAG	CAA	1249
Val	Thr	Pro	Pro	Arg	Leu	Arg	Glu	Lys	Tyr	Asp	His	His	Ala	Gln	Gln	
				405					410					415		
TTC	AAC	TCG	TTC	CAC	TGC	AAC	CCC	GCG	CAT	GAG	AGC	AAC	AGC	CTG	CCC	1297
Phe	Asn	Ser	Phe	His	Cys	Asn	Pro	Ala	His	Glu	Ser	Asn	Ser	Leu	Pro	
			420					425					430			
CCC	AGC	CCC	TTG	AGG	ATA	GTG	GAG	GAT	GAG	GAA	TAT	GAA	ACG	ACC	CAG	1345
Pro	Ser	Pro	Leu	Arg	Ile	Val	Glu	Asp	Glu	Glu	Tyr	Glu	Thr	Thr	Gln	
		435					440					445				
GAG	TAC	GAA	CCA	GCT	CAA	GAG	CCG	GTT	AAG	AAA	CTC	ACC	AAC	AGC	AGC	1393
Glu	Tyr	Glu	Pro	Ala	Gln	Glu	Pro	Val	Lys	Lys	Leu	Thr	Asn	Ser	Ser	
	450					455					460					
CGG	CGG	GCC	AAA	AGA	ACC	AAG	CCC	AAT	GGT	CAC	ATT	GCC	CAC	AGG	TTG	1441
Arg	Arg	Ala	Lys	Arg	Thr	Lys	Pro	Asn	Gly	His	Ile	Ala	His	Arg	Leu	
465					470					475					480	
GAA	ATG	GAC	AAC	AAC	ACA	GGC	GCT	GAC	AGC	AGT	AAC	TCA	GAG	AGC	GAA	1489
Glu	Met	Asp	Asn	Asn	Thr	Gly	Ala	Asp	Ser	Ser	Asn	Ser	Glu	Ser	Glu	
				485					490					495		
ACA	GAG	GAT	GAA	AGA	GTA	GGA	GAA	GAT	ACG	CCT	TTC	CTG	GCC	ATA	CAG	1537
Thr	Glu	Asp	Glu	Arg	Val	Gly	Glu	Asp	Thr	Pro	Phe	Leu	Ala	Ile	Gln	
			500					505					510			
AAC	CCC	CTG	GCA	GCC	AGT	CTC	GAG	GCG	GCC	CCT	GCC	TTC	CGC	CTG	GTC	1585
Asn	Pro	Leu	Ala	Ala	Ser	Leu	Glu	Ala	Ala	Pro	Ala	Phe	Arg	Leu	Val	
		515					520					525				
GAC	AGC	AGG	ACT	AAC	CCA	ACA	GGC	GGC	TTC	TCT	CCG	CAG	GAA	GAA	TTG	1633
Asp	Ser	Arg	Thr	Asn	Pro	Thr	Gly	Gly	Phe	Ser	Pro	Gln	Glu	Glu	Leu	
	530					535					540					
CAG	GCC	AGG	CTC	TCC	GGT	GTA	ATC	GCT	AAC	CAA	GAC	CCT	ATC	GCT	GTC	1681
Gln	Ala	Arg	Leu	Ser	Gly	Val	Ile	Ala	Asn	Gln	Asp	Pro	Ile	Ala	Val	
545					550				555						560	
TAAAACCGAA	ATACACCCAT	AGATTCACCT	GTAAACTTT	ATTTTATATA	ATAAAGTATT											1741
CCACCTTAAA	TTAAACAAAA	AAA														1764

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FIG. 17A
(SEQ ID NOS:51-52)

CAT His 1	CAA Gln	GTG Val	TGG Trp	GCG Ala	GCG Ala	AAA Lys	GCC Ala	GGG Gly	GGC Gly	TTG Leu	AAG Lys	AAG Lys	GAC Asp	TCG Ser	CTG Leu	48
CTC Leu	ACC Thr	GTG Val	CGC Arg	CTG Leu	GGC Gly	GCC Ala	TGG Trp	GGC Gly	CAC His	CCC Pro	GCC Ala	TTC Phe	CCC Pro	TCC Ser	TGC Cys	96
GGG Gly	CGC Arg	CTC Leu	AAG Lys	GAG Glu	GAC Asp	AGC Ser	AGG Arg	TAC Tyr	ATC Ile	TTC Phe	TTC Phe	ATG Met	GAG Glu	CCC Pro	GAG Glu	144
GCC Ala	AAC Asn	AGC Ser	AGC Ser	GGC Gly	GGG Gly	CCC Pro	GGC Gly	CGC Arg	CTT Leu	CCG Pro	AGC Ser	CTC Leu	CTT Leu	CCC Pro	CCC Pro	192
TCT Ser	CGA Arg	GAC Asp	GGG Gly	CCG Pro	GAA Glu	CCT Pro	CAA Gln	GAA Glu	GGA Gly	GGT Gly	CAG Gln	CCG Pro	GGT Gly	GCT Ala	GTG Val	240
CAA Gln	CGG Arg	TGC Cys	GCC Ala	TTG Leu	CCT Pro	CCC Pro	CGC Arg	TTG Leu	AAA Lys	GAG Glu	ATG Met	AAG Lys	AGT Ser	CAG Gln	GAG Glu	288
TCT Ser	GTG Val	GCA Ala	GGT Gly	TCC Ser	AAA Lys	CTA Leu	GTG Val	CTT Leu	CGG Arg	TGC Cys	GAG Glu	ACC Thr	AGT Ser	TCT Ser	GAA Glu	336
TAC Tyr	TCC Ser	TCT Ser	CTC Leu	AAG Lys	TTC Phe	AAG Lys	TGG Trp	TTC Phe	AAG Lys	AAT Asn	GGG Gly	AGT Ser	GAA Glu	TTA Leu	AGC Ser	384
CGA Arg	AAG Lys	AAC Asn	AAA Lys	CCA Pro	GAA Glu	AAC Asn	ATC Ile	AAG Lys	ATA Ile	CAG Gln	AAA Lys	AGG Arg	CCG Pro	GGG Gly	AAG Lys	432
TCA Ser	GAA Glu	CTT Leu	CGC Arg	ATT Ile	AGC Ser	AAA Lys	GCG Ala	TCA Ser	CTG Leu	GCT Ala	GAT Asp	TCT Ser	GGA Gly	GAA Glu	TAT Tyr	480
ATG Met	TGC Cys	AAA Lys	GTG Val	ATC Ile	AGC Ser	AAA Lys	CTA Leu	GGA Gly	AAT Asn	GAC Asp	AGT Ser	GCC Ala	TCT Ser	GCC Ala	AAC Asn	528

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FIG. 17B

ATC ACC ATT GTG GAG TCA AAC GCC ACA TCC ACA TCT ACA GCT GGG ACA Ile Thr Ile Val Glu Ser Asn Ala Thr Ser Thr Ser Thr Ala Gly Thr 180 185 190	576
AGC CAT CTT GTC AAG TGT GCA GAG AAG GAG AAA ACT TTC TGT GTG AAT Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn 195 200 205	624
GGA GGC GAG TGC TTC ATG GTG AAA GAC CTT TCA AAT CCC TCA AGA TAC Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr 210 215 220	672
TTG TGC AAG TGC CAA CCT GGA TTC ACT GGA GCG AGA TGT ACT GAG AAT Leu Cys Lys Cys Gln Pro Gly Phe Thr Gly Ala Arg Cys Thr Glu Asn 225 230 235 240	720
GTG CCC ATG AAA GTC CAA ACC CAA GAA AAG TGC CCA AAT GAG TTT ACT Val Pro Met Lys Val Gln Thr Gln Glu Lys Cys Pro Asn Glu Phe Thr 245 250 255	768
GGT GAT CGC TGC CAA AAC TAC GTA ATG GCC AGC TTC TAC AGT ACG TCC Gly Asp Arg Cys Gln Asn Tyr Val Met Ala Ser Phe Tyr Ser Thr Ser 260 265 270	816
ACT CCC TTT CTG TCT CTG CCT GAA TAGCGCATCT CAGTCGGTGC CGCTTTCTTG Thr Pro Phe Leu Ser Leu Pro Glu 275 280	870
TTGCCGCATC TCCCCTCAGA TTCCNCCTAG AGCTAGATGC GTTTTACCAG GTCTAACATT	930
GACTGCCTCT GCCTGTCGCA TGAGAACATT AACACAAGCG ATTGTATGAC TTCCTCTGTC	990
CGTGACTAGT GGGCTCTGAG CTA CTCTCGTAG GTGCGTAAGG CTCCAGTGTT TCTGAAATTG	1050
ATCTTGAATT ACTGTGATAC GACATGATAG TCCCTCTCAC CCAGTGCAAT GACAATAAAG	1110
GCCTTGAAAA GTCAAAAAA AAAAAAAAAA	1140

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FIG. 18A
(SEQ ID NOS:53-54)

AGTTTCCCCC CCCAACTTGT CGGAACTCTG GGCTCGCGCG CAGGGCAGGA GCGGAGCGGC	60
GGCGGCTGCC CAGGCGATGC GAGCGCGGGC CGGACGGTAA TCGCCTCTCC CTCCTCGGGC	120
TGCGAGCGCG CCGGACCGAG GCAGCGACAG GAGCGGACCG CGGCGGGAAC CGAGGACTCC	180
CCAGCGGCGC GCCAGCAGGA GCCACCCCGC GAGCGTGCGA CCGGGACGGA GCGCCCGCCA	240
GTCCCAGGTG GCGCGGACCG CACGTTGCGT CCGCGCGCTC CCGCGCGGCG ACAGGAGACG	300
CTCCCCCCCCA CGCGCGCGCG GCCTCGGCCC GGTCGCTGGC CCGCCTCCAC TCGGGGACA	360
AACTTTTCCTC GAAGCCGATC CCAGCCCTCG GACCCAACT TGTCGCGCGT CGCCTTCGCC	420
GGGAGCCGTC CGCGCAGAGC GTGCACTTCT CGGGCGAG ATG TCG GAG CGC AGA	473
Met Ser Glu Arg Arg	
1 5	
GAA GGC AAA GGC AAG GGG AAG GGC GGC AAG AAG GAC CGA GGC TCC GGG	521
Glu Gly Lys Gly Lys Gly Lys Gly Lys Lys Lys Asp Arg Gly Ser Gly	
10 15 20	
AAG AAG CCC GTG CCC GCG GCT GGC GGC CCG AGC CCA GCC TTG CCT CCC	569
Lys Lys Pro Val Pro Ala Ala Gly Gly Pro Ser Pro Ala Leu Pro Pro	
25 30 35	
CGC TTG AAA GAG ATG AAG ATG CAG GAG TCT GTG GCA GGT TCC AAA CTA	617
Arg Leu Lys Glu Met Lys Ser Gln Glu Ser Val Ala Gly Ser Lys Leu	
40 45 50	
GTG CTT CGG TGC GAG ACC AGT TCT GAA TAC TCC TCT CTC AAG TTC AAG	665
Val Leu Arg Cys Glu Thr Ser Ser Glu Tyr Ser Ser Leu Lys Phe Lys	
55 60 65	
TGG TTC AAG AAT GGG AGT GAA TTA AGC CGA AAG AAC AAA CCA CAA AAC	713
Trp Phe Lys Asn Gly Ser Glu Leu Ser Arg Lys Asn Lys Pro Gln Asn	
70 75 80 85	
ATC AAG ATA CAG AAA AGG CCG GGG AAG TCA GAA CTT CGC ATT AGC AAA	761
Ile Lys Ile Gln Lys Arg Pro Gly Lys Ser Glu Leu Arg Ile Ser Lys	
90 95 100	
GCG TCA CTG GCT GAT TCT GGA GAA TAT ATG TGC AAA GTG ATC AGC AAA	809
Ala Ser Leu Ala Asp Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys	
105 110 115	

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FIG. 18B

CTA GGA AAT GAC AGT GCC TCT GCC AAC ATC ACC ATT GTG GAG TCA AAC Leu Gly Asn Asp Ser Ala Ser Ala Asn Ile Thr Ile Val Glu Ser Asn 120 125 130	857
GAG ATC ACC ACT GGC ATG CCA GCC TCA ACT GAG ACA GCG TAT GTG TCT Glu Ile Thr Thr Gly Met Pro Ala Ser Thr Glu Thr Ala Tyr Val Ser 135 140 145	905
TCA GAG TCT CCC ATT AGA ATA TCA GTA TCA ACA GAA GGA ACA AAT ACT Ser Glu Ser Pro Ile Arg Ile Ser Val Ser Thr Glu Gly Thr Asn Thr 150 155 160 165	953
TCT TCA TCC ACA TCC ACA TCT ACA GCT GGG ACA AGC CAT CTT GTC AAG Ser Ser Ser Thr Ser Thr Ser Thr Ala Gly Thr Ser His Leu Val Lys 170 175 180	1001
TGT GCA GAG AAG GAG AAA ACT TTC TGT GTG AAT GGA GGC GAG TGC TTC Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu Cys Phe 185 190 195	1049
ATG GTG AAA GAC CTT TCA AAT CCC TCA AGA TAC TTG TGC AAG TGC CCA Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu Cys Lys Cys Pro 200 205 210	1097
AAT GAG TTT ACT GGT GAT CGC TGC CAA AAC TAC GTA ATG GCC AGC TTC Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn Tyr Val Met Ala Ser Phe 215 220 225	1145
TAC AGT ACG TCC ACT CCC TTT CTG TCT CTG CCT GAA TAGGCGCATG Tyr Ser Thr Ser Thr Pro Phe Leu Ser Leu Pro Glu 230 235 240	1191
CTCAGTCGGT GCCGCTTTCT TGTTGCCGCA TCTCCCCTCA GATTCAACCT AGAGCTAGAT	1251
GCGTTTTACC AGGTCTAACA TTGACTGCCT CTGCCTGTG CATGAGAACA TTAACACAAG	1311
CGATTGTATG ACTTCCTCTG TCCGTGACTA GTGGGCTCTG AGCTACTCGT AGGTGCGTAA	1371
GGCTCCAGTG TTTCTGAAAT TGATCTTGAA TTACTGTGAT ACGACATGAT AGTCCCTCTC	1431
ACCCAGTGCA ATGACAATAA AGGCCTTGAA AAGTCTCACT TTTATTGAGA AAATAAAAAAT	1491
CGTTCCACGG GACAGTCCCT CTTCTTTATA AAATGACCCT ATCCTTGAAA AGGAGGTGTG	1551
TTAAGTTGTA ACCAGTACAC ACTTGAAATG ATGGTAAGTT CGCTTCGGTT CAGAATGTGT	1611
TCTTTCTGAC AAATAAACAG AATAAAAAAA AAAAAAAAAA A	1652

INTERNATIONAL SEARCH REPORT

International application No.

US98/21349

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 31/70, 38/00, 38/02, 38/18

US CL : 514/2, 12, 44, 903, 907

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2, 12, 44, 903, 907

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,530,109 A (GOODEARL et al.) 25 June 1996, column 3, line 3 to column 6, line 53 and column 11, line 1 to column 12, line 59.	1-34

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* "A" "E" "L" "O" "P"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance earlier document published on or after the international filing date document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	"T" "X" "Y" "A"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art document member of the same patent family
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Date of the actual completion of the international search

17 DECEMBER 1998

Date of mailing of the international search report

05 FEB 1999

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
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INTERNATIONAL SEARCH REPORT

International application No.

/US98/21349

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 35-36
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/ISA/21349

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, MEDLINE, SCISEARCH, EMBASE, BIOSIS, CAPLUS, WPIDS, BIOTECHDS, CONFSCI, LIFESCI
neuregulin#, glia#, heregulin#, GGF#, ischemia#, dementia#, Parkinson#, Huntington#, Alzheimer#, infarct#,
amyotrophic, Down#, Korsakoff#, heart#, cardiac, spinal